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P. J. DU TOIT,  
*Director of Veterinary Services and Animal Industry.*

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## Section I.

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# Protozoal Diseases.

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- NETZ, W. O.                      The transmission of *Spirochaeta theileri* to a blesbuck. (*Damaliscus albifrons*).
- NETZ, W. O.                      Bovine anaplasmosis.--The transmission of *Anaplasma marginale* to a black wildebeest (*Conochastes gnu*).



## **The Transmission of *Spirochaeta Theileri* to a Blesbuck (*Damaliscus Albifrons*).**

By W. O. NEITZ, B.V.Sc., Veterinary Research Officer,  
Onderstepoort

The presence of spirochaetes in antelopes was first described by Bruce and his co-workers in a bushbuck (*Tragelaphus scriptus*) in Uganda (1911). Todd and Wolbach (1912) recorded the presence of spirochaetes in a roan antelope (*Hippotragus equinus*) in the Gambia. Spirochaetes were also found by Schwetz and Collart (1929) in blood smears of an antelope (*Cobus vardonii*) in the Belgian Congo.

Dodd (1906) came to the conclusion that the blood spirochaetes found in horses and sheep are identical with *Sp. theileri* of cattle.

In order to ascertain whether antelopes are susceptible to *Sp. theileri* a splenectomized blesbuck which had been experimentally infected with *Anaplasma marginale* was utilized. Blood smears from this animal had been examined daily for a period of nine months and apart from *A. marginale* no other blood parasites were observed.

Blood from a cow in which *Sp. theileri* could be demonstrated microscopically was injected subcutaneously into this blesbuck. From the 5th to the 13th day after injection a fair number of spirochaetes identical with *Sp. theileri* could be demonstrated. The animal did not show any ill effects as result of the infection.

*Conclusion.*---It was possible to infect the blesbuck with *Spirochaeta theileri* from cattle.

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## **Bovine Anaplasmosis: The Transmission of *Anaplasma Marginale* to a Black-Wilde- beest. (*Conochaetes Gnu*).**

By W. O. NEITZ, B.V.Sc., Veterinary Research Officer,  
Onderstepoort.

### INTRODUCTION.

IN 1932 Neitz and du Toit reported on the susceptibility of the blesbuck (*Damaliscus albifrons*) and the duiker (*Sylvicapra grimmia grimmia* L.) to *Anaplasmosis*. Since then it has been found that another South African Antelope, the Black-Wildebeest is also susceptible to *Anaplasma marginale*.

### EXPERIMENTAL OBSERVATIONS.

A black-wildebeest was obtained from the farm Spesbona, Geneva Station, Orange Free State, and arrived at Onderstepoort on the 25th June, 1932. It is not known to what extent ticks occur in that area but on arrival no ticks could be found on the animal. During the time that the animal was in experiment it was kept in an apparently tick free camp. In the beginning the animal was very wild but later became so tame that it would lie down and patiently wait for its temperature to be taken and for smears to be prepared.

#### *Experiment (1). (S. 4762.)*

*Object.*—To ascertain whether it is possible to demonstrate microscopically blood parasites belonging to the families Babesidae, Theileridae and Anaplasmatidae in the black-wildebeest.

*Method.*—Blood-smears were examined twice weekly for a period of six months.

*Result.*—No blood parasites could be demonstrated during this period.

#### *Experiment (2). (S.4953.)*

*Object.*—To transmit *P. bigeminum*, *Th. mutans* and *A. marginale* to the black-wildebeest.

*Method.*—20 c.c. Blood from calf 4660 which harboured *P. bigeminum*, *Th. mutans* and a virulent strain of *A. marginale* was injected subcutaneously into black-wildebeest 5193 (Fig 1).

*Result.*—It was not possible to demonstrate *P. bigeminum* and *Th. mutans* microscopically but on the 38th day after subinoculation *A. marginale* appeared and could be demonstrated for a period of 17 days. Up to 2 per cent. of the erythrocytes were infected. The animal did not manifest any clinical symptoms, no temperature reaction and no anaemic changes in the blood.

#### *Experiment (3).*

*Object.*—(1) To ascertain whether a latent infection of *P. bigeminum*, *Th. mutans* existed in black-wildebeest.

(2) (a) To confirm the diagnosis of *A. marginale*.

(b) To note whether the virulence and morphology of *A. marginale* was changed through passage.

*Method.*—10 c.c. Blood from black-wildbeest was injected subcutaneously into two six-months-old calves 5214 and 5271 reared under tick free conditions.

TABLE.—*Experiments (2) and (3).*

D.O.B. No. of animal.	Date of injection.	Injected from.	Incubation period in days of <i>A. marginale</i> .	Remarks.
Black - wildebeest 5193	1/2/33	4660	38	No clinical symptoms. No anaemic changes.
Calf 5214....	25/4/33	5193	27	Typical anaplasmosis reaction with marked anaemic changes in blood.
Calf 5271....	5/6/33	5193	22	No clinical symptoms. Marked anaemic changes in blood.

*Result.*—*P. bigeminum* and *Th. mutans* could not be demonstrated in the above two calves. After an incubation period of 27 days in case of 5214 and 22 days in case of 5271 *A. marginale* appeared. Both animals showed temperature reactions and marked anaemic changes. Calf 5214 showed listlessness, inappetence and constipation and was treated satisfactorily with a purgative. Both calves were found later to be susceptible to *P. bigeminum* and *Th. mutans* when injected with known infective blood.

#### CONCLUSIONS.

1. It was found that the black-wildebeest is susceptible to *A. marginale* but no clinical symptoms were noticed.

2. Neither *P. bigeminum* nor *Th. mutans* could be transmitted to the black-wildebeest.

3. *A. marginale* neither lost its virulence nor its characteristic morphology by passage through the black-wildebeest.

4. The fact that the black-wildebeest is susceptible to *A. marginale* but refractive to *P. bigeminum* and *Th. mutans*, indicates that this antelope can also be utilized for separating these three parasites which usually occur together in South Africa and thus obtaining pure infection of *A. marginale*.

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- NEITZ, W. O., AND DU TOIT, P. J. (1932). Bovine Anaplasmosis: A Method of obtaining Pure Strains of *Anaplasma marginale* and *Anaplasma centrale* by transmission through antelopes. 18th Rep. Dir. Vet. Serv. and An. Ind. Union of South Africa, pp. 3-20.

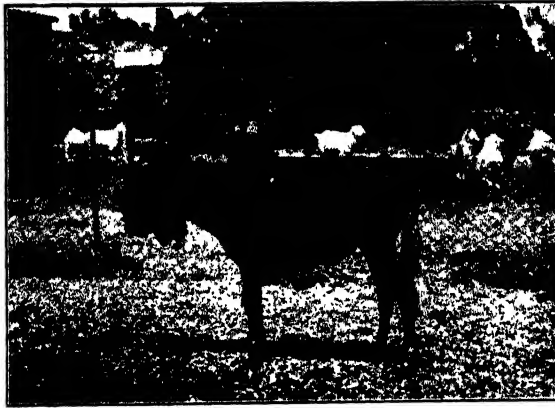


Fig. 1.—Black Wildebeest 5193, harbouring *A. marginale*.



## Section II.

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# Virus Diseases.

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- ALEXANDER, R. A., AND    The transmission of louping ill by ticks  
NETZ, W. O.                (*Rhipicephalus appendiculatus*).
- NETZ, W. O. . . . .    The blesbuck (*Damaliscus Albifrons*) and  
                                 the black wildebeest (*Conochaetes gnu*)  
                                 as carriers of heartwater.



## **The Transmission of Louping Ill by Ticks (*Rhipicephalus appendiculatus*).**

R. A. ALEXANDER, B.Sc. Agric., D.V.Sc., Empire Marketing  
Board Research Fellow;

W. O. NEITZ, B.V.Sc., Veterinary Research Officer, Onderstepoort.

IN July, 1933, a preliminary report was published on the transmission of Louping Ill to sheep by *R. appendiculatus* nymphae that had picked up the virus infection by feeding on a reacting sheep in the previous larval stage. These initial results prompted a fairly extensive study of the mode of transmission of this virus by the brown tick. The outcome of this work is published in the hope that it may be of some value to workers in those areas where the disease is known to occur, in addition to being useful, should the disease be diagnosed in this country at some future date. In this connection it must be pointed out that a thorough knowledge of the infectivity of the various stages of a species of tick, after feeding on a variety of hosts, is not only of academic interest, but is of extreme practical importance because this knowledge must serve as a basis for the promulgation of those prophylactic measures which aim at the eradication of the disease by elimination of the infective arthropod vector. Bearing this in mind it became essential to determine the susceptibility of some domestic animals, other than sheep, which might serve as hosts and potential reservoirs of infection for the ticks. Therefore, while the tick breeding work was in progress, steps were taken to determine the susceptibility of cattle and of horses to Louping Ill.

### **THE SUSCEPTIBILITY OF CATTLE TO LOUPING ILL.**

On 7th October, 1932, a calf (4535) was injected intrathecally with 2 c.c. of the turbid supernatant fluid obtained from centrifuging a 1 per cent. saline emulsion of desiccated infective mouse brain. The operation was performed with full aseptic precautions under chloral hydrate anaesthesia by inserting a long fine trocar and canula between the atlas and axis into the spinal canal.

On the afternoon of the third day after injection the temperature rose abruptly to  $105\cdot3^{\circ}$ , but no clinical symptoms could be detected. The following morning the temperature was  $105\cdot6^{\circ}$ , there was complete inappetence, the abdomen was markedly tucked up and there were signs of profuse watery diarrhoea. Salivation was fairly pronounced, respirations hurried and shallow and the animal either wandered aimlessly in an arch round the box, or stood in a semi stupor with wild, staring eyes and twitching muscles. During the course



of the day several violent fits, characterized by wild charging round the box, occurred. Towards evening these fits became much more frequent (2—3 per hour) and could be initiated by giving the animal a fright say by suddenly shouting or clapping the hands. Vision was now markedly impaired since the calf would charge, bellowing, with its head down into the walls or the manger. It would continue its mad stampede for several minutes before falling to the ground completely exhausted in a semi comatose condition. After some time it would recover sufficiently to stagger to its feet and after staring into space with a vacant expression would commence another wild charge. Any attempt at control was quite impossible and severe abrasions of the head, the supraorbital crest and the horns were sustained as a result of crashing into the walls. Later in the evening a paresis of the hindquarters developed so that the wild charging became less vigorous. The following morning the body temperature had fallen to 102·6 and the animal was unable to raise its hindquarters off the ground. Paralysis progressed rapidly from behind cranially so that by mid-day even a sternal position could not be maintained. The animal remained on its side but retained full consciousness as evidenced by flicking of the ears to chase away flies and obvious recognition of the approach of an attendant. Death occurred the same afternoon presumably as a result of paralysis of the diaphragm.

Post-mortem examination revealed no marked abnormality other than the severe abrasions due to traumata. The meningeal vessels showed slight engorgement but there was no evidence of a purulent meningitis or abscessation of the brain.

The brain was removed with aseptic precautions and stored overnight in the freezing chamber of an electric refrigerator. Next morning the surface of one cerebral hemisphere was washed with sterile saline and a small portion about 1 cm. square excised from the depth of the brain substance. A 1 per cent. saline emulsion of this material was injected intracerebrally in 0·03 c.c. amounts into each of three mice; all the mice died on the 6th day after showing typical symptoms of Louping Ill. This indicates that a considerable penetration and probably a considerable multiplication of the virus had taken place. The diagnosis of Louping Ill was confirmed by injecting 1 c.c. of a 0·2 per cent. emulsion of the brains of the mice subcutaneously into two susceptible sheep. Both sheep succumbed to typical Louping Ill.

In an attempt to confirm the susceptibility of cattle to the disease numerous *R. appendiculatus* nymphae from a batch (1297 B.c) known to be infected with Louping Ill virus were fed on the ears of a nine months old calf (5251). The ticks attached on 9.11.33 and all had engorged and detached by 17.11.33. Contrary to expectations the animal showed no febrile or clinical reaction, and after an interval of 17 days about 200 nymphae of the same batch were fed on the same calf. No reaction followed. Therefore, as an immunity test 42 days later a subcutaneous injection of 5 c.c. of a 1 per cent emulsion of infective mouse brain was given; at the same time two sheep and an additional calf (5412) were given a similar injection to serve as controls. Both sheep reacted typically and recovered. Both calves failed to exhibit any reaction. Two months later the original

calf (5251) and an additional heifer (5410) were given an intrathecal infection of 3 c.c. of a 1:300 saline emulsion of fresh infective mouse brain. The first calf showed no reaction. The temperature record of the control is shown in figure 1.

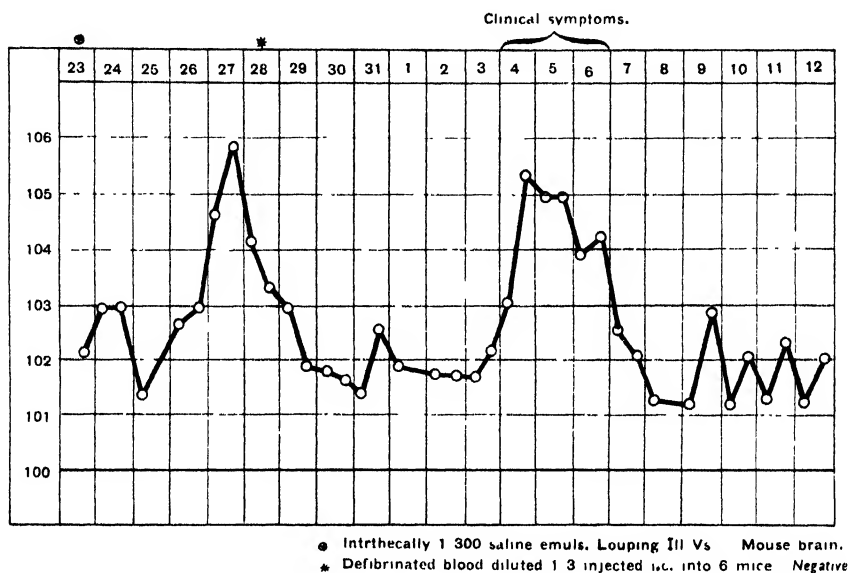


Fig. 1.

It will be noted that a diphasic febrile reaction occurred. During the first febrile period no symptoms of any kind were observed. During the second exacerbation the animal appeared somewhat excitable and nervous. There was spasmodic twitching of the muscles of the thighs, shoulders and neck. The ears continually twitched and there was a peculiar nodding of the head. These symptoms were noticed for about three days after which the habitus returned to normal.

An attempt was made to demonstrate the presence of circulating virus in the blood on 28.3.34 and 5 and 6.4.34, i.e. at the height of each febrile reaction, by the injection of defibrinated blood intracerebrally into mice (0.03 c.c.) and subcutaneously into sheep (5 c.c. subcut.). No virus could be detected.

During the course of the above experiments serum had been collected from the animals as follows:—

- A. 5251 on 23.3.34, i.e. after the tick feeding and subcutaneous injection but prior to the intrathecal injection.
- B. 5412 on 24.3.34, i.e. 65 days after the subcutaneous injection.
- C. 5410 on 19.4.34, i.e. 30 days after the intrathecal injection.

In addition serum was obtained from a normal heifer, from a normal sheep and from a sheep which had recently recovered from an attack of Louping Ill initiated by a subcutaneous injection of virus.

# TRANSMISSION OF LOUPING ILL BY TICKS.

In vitro neutralization tests were set up with these sera using the following technique. To each of a series of agglutination tubes 1 c.c. of five-fold dilutions of a stock emulsion of infective brain in 1/10 horse-serum-saline was added, the dilutions varying from 1/5 to 1:5<sup>9</sup>. The sera to be tested for neutralising antibodies were diluted 1:3 in saline and added to the respective virus dilutions in 1 c.c. amounts. After incubation at 37° for two hours and overnight fixation in the ice box mice were injected with the following results.

Sera diluted 1:3 in saline.	Five-fold dilutions of stock virus emulsion in 1/10 horse-serum-saline.								
	1:5	1:5 <sup>2</sup>	1:5 <sup>3</sup>	1:5 <sup>4</sup>	1:5 <sup>5</sup>	1:5 <sup>6</sup>	1:5 <sup>7</sup>	1:5 <sup>8</sup>	1:5 <sup>9</sup>
Calf 5251 - A.....	7:8	7:7	7:0	0:0	0:0	0:0	0:0	0:0	0:0
5412 = B.....	6:7	8:9	5:8	0:0	0:0	0:0	0:0	0:0	0:0
5410 = C.....	7:7	9:0	9:0	0:0	0:0	0:0	0:0	0:0	0:0
Normal sheep.....	6:6	6:6	6:6	6:7	6:6	7:8	0:0	0:0	0:0
Normal calf.....	6:6	6:6	6:6	6:6	6:7	7:8	7:0	0:0	0:0
Immune sheep.....	7:7	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0

NOTE.—The numeral denotes the number of days after injection on which the injected mouse died. 0 indicated mouse still alive 14 days after injection. Two mice used for each solution and 0.05 c.c. injected intracerebrally, thus 7:8 means two mice injected of which one died on day 7 and one on day 8.

A consideration of the table indicates that after contact with normal calf or sheep serum the stock virus emulsion used was infective for mice in doses of 0.05 c.c. in a dilution of 1:5<sup>9</sup>. After neutralization in vitro by the sera of the bovines being tested the same virus was infective in a dilution of 1:5<sup>2</sup> or 1:5<sup>3</sup>. This indicates the presence of virucidal antibodies in the sera in a fairly high concentration though not so high as encountered in the serum of an immune sheep used as a control.

*Conclusions.*—From this series of experiments it must be concluded that the intrathecal injection of the virus of Louping Ill into bovines is followed by a febrile reaction which may or may not be accompanied by clinical symptoms of varying severity and may or may not be fatal. With recovery virucidal antibodies make their appearance in the serum. After subcutaneous injection of virus or the feeding of infective ticks no demonstrable reaction is to be expected but immune bodies are developed.

It is realized that a considerable amount of additional work is necessary to ascertain the full susceptibility of bovines and to determine whether there is a multiplication and circulation of virus in the blood stream after injection, but it is not unreasonable to believe that cattle may serve as a potential reservoir of infection for ticks in the field.

## THE SUSCEPTIBILITY OF THE HORSE.

On 31st October, 1932, a horse (20308) was infested on the ears with numerous *R. appendiculatus* nymphae which had fed on a reacting sheep (34115) as larvae and represented part of a batch (1138 Ac) which were known to be infected with the virus of Louping Ill. The ticks attached readily and engorged well. As soon as they commenced to drop three individuals were emulsified in 5 c.c. of saline and injected subcutaneously into one sheep (34191). This sheep developed typical Louping Ill and was destroyed in extremis. The horse did not show any departure from normal health and after an interval of 18 days was given an intrathecal injection of 2 c.c. of a 0.5 per cent. emulsion of infective mouse brain. Three control mice injected intracerebrally with 0.05 c.c. of the same emulsion died on the 5th and 6th day. The horse showed no febrile reaction but after an interval of 14 days developed a slight posterior paresis which persisted for several weeks. At no time was it possible to associate the condition with Louping Ill since blood drawn on various occasions from the 7th day after injection was constantly non-infective for mice. On 17th March, 1933, the horse was again infested on the ears with nymphae known to contain virus, but no constitutional disturbance was produced in the host after engorgement.

The results obtained from this one horse were so inconclusive that a second horse (20374) was infested on the ears with *R. appendiculatus* nymphae which had fed as larvae on a sheep (37412) during a Louping Ill reaction produced by subcutaneous injection of mouse virus. On the 5th day after infestation there occurred a slight rise in temperature to  $102.2^{\circ}$ , which persisted for 36 hours. On the 7th day the temperature rose abruptly to  $104^{\circ}$  and persisted at or above this level for 3 days after which it returned to normal by crisis. During this time the horse was markedly tucked up and showed a cyanotic, dirty mucous membrane; the respirations were hurried and shallow, the pulse exceedingly weak, rapid and thready; there was complete inappetence and a profuse diarrhoea. Recovery was uninterrupted and there were no nervous complications. Daily examination of blood smears stained by May-Grünwald-Giemsa failed to reveal the presence of any parasites, but a moderate monocytosis was apparent. Diffrinated blood drawn on the 8th day after tick infestation, i.e. at the height of the second febrile exacerbation produced typical Louping Ill in four mice all of which died on the 6th day after intracerebral injection. In addition 5 c.c. of the same blood injected subcutaneously into a sheep produced fatal Louping Ill, death occurring on the 8th day. The presence of virus could not be demonstrated in blood drawn two days later, either by intracerebral injection of mice or the subcutaneous injection of a sheep in 2.5 c.c. amount.

On 7th December, 1933 and 21st March, 1934, i.e. 57 and 140 days respectively after tick infestation blood was withdrawn from the horse and subsequently in vitro neutralization tests were carried out with the serum at the same time as those cited for the bovines above. The results obtained are given in Table II.

TABLE II.

*In vitro neutralization of Louping Ill Virus by Serum of Horse 20374.*

Sera diluted 1:3 in saline.	Five fold dilutions of stock virus emulsion in 1/10 horse-serum-saline.								
	1:5	1:5 <sup>2</sup>	1:5 <sup>3</sup>	1:5 <sup>4</sup>	1:5 <sup>5</sup>	1:5 <sup>6</sup>	1:5 <sup>7</sup>	1:5 <sup>8</sup>	1:5 <sup>9</sup>
Serum of 7/12/33...	7:8	7:9	7:0	0:0	0:0	0:0	0:0	0:0	0:0
Serum of 21/3/34...	6:7	7:8	11:11	0:0	0:0	0:0	0:0	0:0	0:0
Serum of normal sheep	6:6	6:6	6:6	6:7	6:6	7:8	0:0	0:0	0:0

It will be seen that virucidal antibodies to a fairly high titre had developed. Most unfortunately serum had not been collected from the first horse (20308) for inclusion in the test and in the meantime the animal had been destroyed.

*Conclusions.*—From this single positive case it may be concluded that the horse is susceptible to Louping Ill, and that after engorgement of infective ticks there is a multiplication of virus whose presence may be demonstrated in the blood stream.

No adequate explanation can be advanced for the failure of the first horse to react to the infestation of known infective ticks. It is suggested, however, that an „infection inapparente” actually did occur, and that this produced an immunity sufficient to withstand the intrathecal injection of virulent brain emulsion.

Attention must be directed to the general similarity of the results obtained with the attempted infection of horses and cattle. No effort has been made to work out the course and nature of the disease in these species of animals, since the simple demonstration of susceptibility and the production of immune bodies in the serum was adequate for our purpose.

## TICK TRANSMISSION.

### A. TECHNIQUE.

For the sake of completeness it is considered necessary to give a short resumé of the methods employed at this Institution for routine tick breeding work.

During the summer months undipped cattle running on the farm Kaalplaas are inspected periodically and fully engorged female ticks are removed carefully. After identification they are placed in test tubes lightly stoppered with cotton wool. The tubes, suitably labelled, are placed in cylindrical glass jars about 22 c.m. high and 14 c.m. in diameter, in the bottom of each of which there is a small quantity of clean sand about 1 c.m. deep, kept moistened with a saturated solution of common salt in water. The jars are placed on

the shelves of a cupboard in a room which happens to be fairly cool in summer and warm in winter. All the jars of ticks are inspected twice or three times weekly. Tubes containing numbers of dead individuals overgrown with moulds are discarded. As soon as oviposition, hatching or moulting from one stage to the next is noticed the date is recorded. It is found unnecessary to change ticks from one tube to another even though the number in a tube, particularly in the case of larvae, appear to be excessive.

Larvae are not fed until they have been hatched a week and subsequent stages are not fed until 10-14 days after moulting. For feeding purposes the ticks are shaken out of the tube into a stout calico bag fitted with a running noose at the mouth and having several lengths of tape attached to facilitate tying to the host. The bags are conveniently filled over a tray placed in a large sink containing disinfectant so that any ticks which escape may be captured or destroyed. Larvae are picked up by means of a camel hair brush and nymphae and adults are handled easily with forceps.

The bags are then placed over the ears or scrotum of the selected host. Sheep should have the area round the ears clipped short to minimize the risk of escape of ticks and the bags should be tied on fairly tightly since the passive hyperaemia induced seems to facilitate engorgement. For the first 48 hours the bags are left undisturbed. After that time the bags are changed daily; ticks which have failed to attach are destroyed; engorged ticks that have dropped to the bottom of the bag are collected and placed in tubes as before, about 100 engorged larvae, 50 engorged nymphae and a single engorged female per tube.

Except on rare occasions beyond the scope of this article no difficulty has been experienced in feeding and breeding ticks by this method and in the case of *Rhipicephalus appendiculatus* used in these experiments the procedure was highly satisfactory.

#### B. TRANSMISSION OF LOUPING ILL FROM LARVAE TO NYMPHAE.

On 31st August, 1932, 5 c.c. of a 1/500 saline emulsion of desiccated infective sheep brain was injected subcutaneously into a sheep (34115). On the third day, immediately the first rise in the febrile temperature curve was noticed, about 1/4 of the progeny of one clean *R. appendiculatus* female were placed on each ear. The larvae fed well and about 800 engorged specimens were collected between the 8th and the 12th day. The larvae commenced their moult to nymphae on 29.9.32.

On 7th October, 1932, a sheep (32241) was infested on one ear with a total of about 80 of these nymphae. The nymphae attached readily but the rate of engorgement was variable so that engorged nymphae continued to drop from 14 to 24.10.32. On the 5th day after tick infestation the temperature of the sheep rose abruptly to 106° F. The sheep showed no nervous symptoms of Louping Ill but the diphasic temperature curve was typical and blood drawn on the second day of fever proved highly infective for mice.

## TRANSMISSION OF LOUPING ILL BY TICKS.

This experiment was repeated on three occasions with nymphae from the same batch (1238 A). One sheep (34787) infested on 19.10.32 commenced to react on the 4th day and died on the 10th day after showing typical nervous symptoms; a second sheep (34894) infested 26.1.33 showed a very mild reaction from the 5th day and recovered; a third sheep (26647) infested on 3.5.33 commenced a severe reaction on the 4th day, and after showing slight nervous symptoms recovered. These two sheep were given an immunity test consisting of the subcutaneous injection of 5 c.c. of a 1 per cent. emulsion of infective mouse brain and did not react, whereas two control sheep reacted severely but recovered.

To confirm this transmission from larvae to nymphae the entire experiment was repeated. A sheep (37412) was infested on the ears with numerous clean larvae (batch 1297) on 22nd July, 1933. Two days later a subcutaneous injection of 5 c.c. of a 1 per cent. dilution of infective mouse brain was given. A severe reaction, to which the sheep eventually succumbed, commenced after 48 hours and during the entire febrile reaction numerous engorged larvae continued to drop. These larvae commenced their moult to nymphae on 4th September, 1933. About 80 of these nymphae were fed on the ears of a susceptible sheep (37583) on 28th September, 1933. The ticks attached immediately and produced a mild attack of Louping Ill from the third to the eighth day. After recovery the sheep was solidly immune to the subcutaneous injection of a massive dose of mouse virus, the infectivity being controlled by subcutaneous injection into two controls and into mice.

*Conclusion.*—*Rhipicephalus appendiculatus* larvae become infected with the virus of Louping Ill after engorging on an animal undergoing a reaction. The resulting nymphae will pass on this infection when feeding on a susceptible animal.

### C. TRANSMISSION FROM NYMPHAE TO ADULTS.

The nymphae used to pick up the infection in this experiment were obtained from larvae which had been fed on one of the susceptible calves used as routine tick feeders. At no time had there been any possible association with Louping Ill.

About 50 nymphae were placed on one ear of a sheep (32241) as soon as the first rise in temperature occurred after infective nymphae had been fed on the other ear. The ticks attached readily and were collected fully engorged from the 6th to the 10th day. At the time the ticks commenced to feed the presence of virus in the peripheral blood was demonstrated by the intracerebral injection of mice. By the time engorgement was complete virus could not be detected but specific immune bodies were demonstrable by in vitro neutralization of mouse virus.

On 14th November, 1932, the nymphae commenced their moult to adults and eleven days later 15 adults were placed on the ears of a susceptible sheep (34660). For some reason all the ticks except a single male refused to attach and eventually died in the ear bags, the sheep showing no deviation from normal health.

Subsequently, on 13th January, 1933, the same sheep was infested with ten adults from the same batch. On this occasion attachment and engorgement were rapid. A typical, severe febrile reaction commenced on the 4th day and lasted for six days. The sheep recovered and five weeks later was found to be immune to the subcutaneous injection of a test dose of virus which proved fatal to two controls.

*Conclusion.*—*R. appendiculatus* nymphae are capable of picking up the virus of Louping Ill while feeding on a reacting sheep, and of transmitting it to a susceptible sheep during engorgement as adults.

#### D. TRANSMISSION FROM ADULTS THROUGH THE EGG TO LARVAE OF THE NEXT GENERATION.

It is unnecessary to detail all the attempts that have been made to determine the possibility of transmission of the virus of Louping Ill through the egg from one generation to the next since every experiment has yielded negative results. Adults which had produced Louping Ill after picking up the infection as nymphae, and had engorged on a reacting host while free virus could be demonstrated in the peripheral blood, laid eggs which were found to be devoid of virus, and the larvae after hatching failed to transmit the disease. Similarly clean adults were fed on a reacting host and were shown to have ingested virus since an emulsion of a few engorged individuals were fully virulent, but no trace of virus could be found in either the eggs or the larvae after hatching.

*Conclusion.*—The eggs laid by *R. appendiculatus* adults harbouring the virus of Louping Ill are non-infective. Larvae hatched from these eggs do not transmit the disease. It is concluded that the virus is incapable of passing through the egg from one generation of tick to the next.

#### E. TRANSMISSION FROM LARVAE THROUGH NYMPHAE TO ADULTS.

##### 1. *Nymphae feeding on a susceptible sheep.*

In previous experiments reported above, a collection was made of numerous engorged nymphae which had produced Louping Ill in the sheep upon which they had fed. These ticks were used for the series of experiments to be described under this heading. For instance, one batch of nymphae (1238 Ac.) were fed on a sheep (34787) and produced a fatal case of the disease. The following injections and infestations were carried out with these nymphae or the resulting adults.

1. 24th October, 1932. Sheep 34874 injected subcutaneously with an emulsion of three engorged nymphae, which detached prior to the commencement of the febrile reaction in the host; injection carried out on the day the ticks dropped.

*Result.*—Severe reaction; sheep died 6th November, 1932. Before death blood infective for mice.



2. 26th October, 1932. Sheep 31051 injected subcutaneously with an emulsion of three engorged nymphae which detached after the febrile reaction in the host had commenced. Injection carried out on the day the ticks dropped.

*Result.*—Severe reaction; sheep died 6th November, 1932. Before death blood infective for mice.

3. 5th December, 1932. Sheep 33970 infested on ears with adults derived from engorged nymphae which detached after the commencement of the febrile reaction in the host.

*Result.*—Severe reaction; sheep died on 24th December, 1932.

4. 13th January, 1933. Sheep 34202 injected subcutaneously with an emulsion of two adults derived from nymphae which detached during the febrile reaction in the host.

*Result.*—Severe reaction; sheep died 25th January, 1933.

5. 13th January, 1933. Sheep 34358 infested on ears with adults which detached as nymphae during the febrile reaction in the host.

*Result.*—Severe prolonged reaction. Sheep recovered and was immune to a test dose of virus given subcutaneously 38 days later.

From this series of experiments it would appear that *R. appendiculatus* which pick up an infection as larvae retain that infection through the nymphal to the adult stage after engorging on a susceptible sheep. Two series of confirmatory experiments were carried out on similar lines except that only the moulted adults were fed or emulsified and injected. Of five sheep which were injected with an emulsion of adults none reacted and subsequently all were found to be fully susceptible to a test dose of mouse virus. Of six sheep on which adults were fed (in each case six adults were placed on one ear) four did not react and two underwent mild reactions and recovered. On immunity test the non-reactors were found to be fully susceptible, the reactors solidly immune.

The significance of these results is discussed after considering the results obtained with adults derived from nymphae which fed on an immune sheep.

## *2. Nymphae feeding on an immune sheep.*

The immune sheep (34497) used in this experiment had survived a typical Louping Ill reaction set up by tick infestation and had been shown to be immune to the subcutaneous injection of a massive dose of mouse brain virus.

On 13th January, 1933, numerous *R. appendiculatus* nymphae belonging to the same batch of infective ticks which had produced the disease in experiments cited above were placed in a bag over the

right ear. The ticks engorged readily and detached on the 6th, 7th, and 8th days. No reaction was produced in the sheep. The nymphae commenced their moult to adults on 17 February, 1933, and with the imagines the following experiments were carried out:—

1. 21st March, 1933. Sheep 35019 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction and six weeks later the sheep reacted to an immunity test of mouse brain virus.

2. 21st March, 1933. Sheep 35024 infested on ears with six adults.

*Result.*—Febrile reaction commenced on the 5th day and six weeks later the sheep resisted a test dose of mouse brain virus.

3. 19th April, 1933. Sheep 33110 injected subcutaneously with an emulsion of three adults.

*Result.*—There were two rises in temperature to  $106.2^{\circ}$  and  $106.4^{\circ}$  F. on the afternoon of the third and the fifth day respectively. On immunity test six weeks later the sheep succumbed to typical Louping Ill.

4. 19th April, 1933. Sheep 33116 infested on ears with six adults.

*Result.*—Typical febrile reaction commencing on the third day and six weeks later the sheep resisted a test dose of virus.

To confirm these results the entire experiment was repeated at a later date. A proved immune sheep was used as a host for feeding a different batch of nymphae whose infectivity was controlled by the production of Louping Ill in a susceptible sheep upon which a sample was fed. Two sheep were injected with an emulsion of three adults, the emulsions being prepared about two months after the ticks had moulted. No reaction was produced, and the sheep subsequently proved to be fully susceptible. On two sheep numerous adults were fed; the one developed a mild reaction followed by solid immunity, but the other did not react and was found subsequently to be susceptible.

*Conclusions.*—The results obtained from this series of experiments on feeding infective nymphae on susceptible and immune sheep are not perfectly clear cut but it is justifiable to direct attention to the following points.

1. Nymphae which picked up their infection as larvae still contain virus after the completion of engorgement on a susceptible sheep, and before moulting to adults.

2. The majority of adults derived from infective nymphae which engorged on a susceptible sheep, are infective, but it is not clear whether the nymphae did not clean themselves and then become re-infected by feeding at a time when free virus had made its appearance in the peripheral blood of the host.

## TRANSMISSION OF LOUPING ILL BY TICKS.

3. The majority of adults derived from infective nymphae which fed on immune sheep are capable of transmitting the disease. Since the subcutaneous injection of an emulsion such adults usually does not set up the disease, either the virus titre is very low or the ingestion of blood is necessary to "activate" the virus contained.

### F. TRANSMISSION FROM LARVA THROUGH NYMPHAE TO ADULTS.

#### 1. *Nymphae feeding on a susceptible horse.*

On 31st October, 1932, a horse (20308) was infested on the ears with numerous infected nymphae which represented portion of a batch (1238) which had produced Louping Ill in susceptible sheep regularly.\* The ticks attached readily and commenced to drop fully engorged on 3rd November, 1932. The moult to adults commenced on 30th November, 1932. The following feeding and injection experiments were carried out with these ticks.

1. 5th November, 1932. Sheep 34895 injected subcutaneously with an emulsion of three engorged nymphae, the injection being made two days after dropping.

*Result.*—Severe Louping Ill reaction, the sheep being destroyed in extremis.

2. 13th January, 1933. Sheep 34191 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction. On immunity test after 38 days the sheep proved susceptible and died on 7th March, 1933, after showing nervous symptoms of Louping Ill.

3. 12th December, 1932. Sheep 34653 infested on both ears with numerous adults.

*Result.*—No reaction. After 70 days the sheep was susceptible to a test dose of mouse virus.

These results indicate that adults derived from nymphae which fed on a susceptible horse lose their infection even though the presence of virus may have been detected in the nymphae after engorgement.

From the point of view of the control of the disease in practice this finding is of such importance that it was decided to repeat the experiment on a more comprehensive scale.

A batch of larvae (1297), portion of the progeny of a single female were fed on a reacting sheep (37412) from 22nd to 27th July, 1933. These larvae commenced their moult to nymphae on 4th September, 1933. On 11th October, 1933, about 50 nymphae were placed on each ear of a horse (20374) which developed a febrile reaction, during which the presence of virus in the peripheral blood was demonstrated by the intra-cerebral injection of mice and the subcutaneous injection of sheep.\*

\* c/f. Under heading "susceptibility of the horse" above.

The following feeding and injection experiments were carried out with these ticks.

1. 14th October, 1933. Sheep 37391 injected subcutaneously with an emulsion of six partially engorged nymphae forcibly removed after feeding for three days.

*Result.*—No reaction. On immunity test after 46 days sheep developed a severe reaction but recovered.

2. 16th October, 1933. Sheep 35540 injected subcutaneously with an emulsion of six engorged nymphae which had just detached, i.e. before the febrile reaction in the horse commenced.

*Result.*—No reaction. On immunity test after 46 days sheep developed a severe reaction and recovered.

3. 1st November, 1933. Sheep 36878 injected subcutaneously with an emulsion of six nymphae which had detached before the commencement of the febrile reaction in the horse.

*Result.*—No reaction. On immunity test after 46 days developed a severe reaction and recovered.

4. 27th October, 1933. Sheep 36856 injected subcutaneously with an emulsion of six nymphae which had detached and were collected on the day on which the febrile reaction in the horse commenced.

*Result.*—No reaction. On immunity test after 46 days the sheep developed a mild febrile reaction and recovered.

5. 24th October, 1933. Sheep 35532 injected subcutaneously with six engorged nymphae which dropped after commencement of the febrile reaction in the horse.

*Result.*—Severe reaction; died 9th November, 1933.

6. 1st November, 1933. Sheep 36935 injected subcutaneously with six engorged nymphae which detached after the commencement of the febrile reaction in the horse.

*Result.*—Severe reaction. On immunity test after 46 days the sheep was immune.

The remainder of the nymphae commenced their moult to adults on 23rd November, 1933, and subsequently the following experiments were carried out.

1. 16th January, 1934. Sheep 38204 infested on each ear with five adults which dropped as nymphae before the commencement of the reaction in the horse.

*Result.*—No reaction.

2. 18th January, 1934. Sheep 38215 infested on each ear with five adults which dropped as nymphae after the reaction in the horse had commenced.

*Result.*—Severe febrile reaction from which the sheep recovered.

*Conclusions.*—The results obtained from this series of experiments indicate that most infective *R. appendiculatus* nymphae while feeding on a susceptible horse clean themselves, provided that engorgement is completed before the febrile reaction in the host commences, i.e. before the appearance of circulating virus in the peripheral blood stream. Some nymphae, after rapid engorgement in this way, contain virus when they detach but in the limited number of tests conducted this virus was not carried forward through the moult to the adult stage.

Nymphae which have not completed their feed by the time the febrile reaction commences naturally reacquire infection which is transmitted to the succeeding adults.

## 2. *Nymphae feeding on an immune horse.*

On 17th March, 1933, numerous infected nymphae were placed on the ears of a horse (20308) which had survived an intrathecal injection of 5 c.c. of a 2 per cent. emulsion of infective mouse brain. Engorged nymphae commenced to drop on 22nd March, 1933, and the moult to adults started on 29th April, 1933.

The following injection experiments were carried out with these ticks.

1. 24th March, 1933. Sheep 34510 injected subcutaneously with an emulsion of three engorged nymphae.

*Result.*—Severe reaction. On immunity test after 40 days sheep was immune.

2. 1st April, 1933. Sheep 32501 injected subcutaneously with an emulsion of three engorged nymphae.

*Result.*—Severe reaction. Died 19th April, 1933. Louping Ill.

3. 22nd April, 1933. Sheep 34297 injected subcutaneously with an emulsion of three nymphae just prior to moulting.

*Result.*—Severe reaction. Died Louping Ill 1st May, 1933.

4. 27th April, 1933. Sheep 32951 injected subcutaneously with an emulsion of three adults which had just moulted.

*Result.*—No reaction. On immunity test after 40 days the sheep was fully susceptible.

A feeding experiment with the few remaining adults was attempted but the ticks refused to attach. For this reason and because the results were so interesting the entire experiment was repeated in the following way:—

On 27th February, 1934, numerous infected nymphae were placed on the ear of an immune horse (20374) and a susceptible sheep (37618). The ticks readily attached and engorged well on both animals. The sheep showed a typically severe reaction and was destroyed *in extremis* on the 14th day, thus demonstrating the infectivity of the batch of ticks used. The horse showed no departure

from normal health. The following injection and feeding experiments were carried out with the ticks obtained from the horse, the ticks commencing their moult on 28th March, 1934.

1. 6th March, 1934. Sheep 36881 injected subcutaneously with an emulsion of six nymphae, emulsification and injection being carried out on the day on which the ticks detached.

*Result.*—A very mild reaction followed and on immunity test after an interval of 58 days the sheep was solidly immune to mouse virus.

2. 13th March, 1934. Sheep 38873 injected subcutaneously with an emulsion of six nymphae, emulsification and injection being carried out eight days after detachment of the ticks.

*Result.*—No reaction. On immunity test the sheep was found to be fully susceptible.

3. 9th April, 1934. Sheep 38889 infested on ears with adults.

*Result.*—Severe reaction to which the sheep succumbed on the 13th day.

4. 22nd September, 1934. Sheep 38885 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction. On immunity test after 23 days sheep reacted severely.

5. 4th June, 1934, 27th June, 1934, 28th August, 1934. Sheep were infested on ears with numerous adults.

*Result.*—On no occasion was any reaction produced though subsequent immunity test showed the sheep to be fully susceptible.

*Conclusion.*—From this series of experiments it must be concluded that infective nymphae after engorgement on an immune horse, still contain virus when they detach and drop from the host. The great majority of ticks loose this infection during and after the moult to adults, but an odd individual appears to retain sufficient virus to transmit infection during engorgement as an adult. This conclusion is based upon the finding that on only one out of four attempts did adults transmit infection during feeding, and though emulsions of engorged and moulting nymphae were shown to contain virus on two occasions the injection of emulsified adults produced no reaction.

#### (4. TRANSMISSION FROM LARVAE THROUGH NYMPHAE TO ADULTS.

1. Nymphae feeding on susceptible cattle.
2. Nymphae feeding on immune cattle.

This series of experiments was conducted in the same manner as those detailed for the corresponding work on horses and sheep.

Nymphæ, whose infectivity was controlled by the production of the typical disease after feeding on a susceptible sheep, were placed on the ears of:—

1. A calf 5251 which had been reared under tick free conditions and could not have come into contact with the disease.
2. The same calf after it had survived feeding experiments by which time specific virucidal antibodies had made their appearance in the serum (see above).

With the engorged nymphæ and the resulting adults the following injection and feeding experiments were carried out.

*(a) Engorgement on susceptible calf.*

1. 27th February, 1934. Sheep 37590 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction. On immunity test after 24 days sheep reacted severely and was destroyed in extremis on the 11th day.

2. 27th February, 1934. Sheep 37518 infested on the ears with numerous adults.

*Result.*—No reaction. On immunity test after 24 days sheep eventually recovered from a severe reaction.

3. 9th April, 1934. Sheep 38943 injected subcutaneously with emulsion of three adults.

*Result.*—No reaction. On immunity test after 23 days sheep reacted severely and recovered.

4. 9th April, 1934. Sheep 38933 infested on ears with numerous adults.

*Result.*—No reaction. On immunity test after 23 days sheep reacted severely and died on the 13th day.

*Conclusion.*—Adults derived from infective nymphæ that engorged on a susceptible bovine rid themselves of infection.

*(b) Engorgement on an immune calf.*

1. 27th February, 1934. Sheep 32673 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction. On immunity test after 24 days severe reaction.

2. 27th February, 1934. Sheep 37626 infested on ears with numerous adults.

*Result.*—Severe reaction followed by recovery. On immunity test after 24 days no reaction.

3. 11th April, 1934. Sheep 38937 injected subcutaneously with an emulsion of three adults.

*Result.*—No reaction. On immunity test after 23 days severe reaction.

4. 11th April, 1934. Sheep 38935 infested on ears with numerous adults.

*Result.*—No reaction. On immunity test after 23 days severe reaction.

*Conclusion.*—Infective nymphae after engorgement on an immune bovine may or may not carry the infection through the moult to the adult stage. This result is in keeping with those obtained from feeding on sheep and horses and is discussed below.

#### H. THE INFECTIVITY OF ADULTS FROM "CLEAN" NYMPHAE WHICH ENGORGED ON AN IMMUNE SHEEP SIMULTANEOUSLY WITH INFECTED NYMPHAE.

On 13th January, 1933, numerous *R. appendiculatus* nymphae which had been fed as larvae on one of the routine tick feeding calves were placed on the left ear of an immune sheep (34497). At the same time known infected nymphae were placed on the right ear. Both sets of ticks attached and engorged well and subsequently dropped at approximately the same time. The clean ticks commenced their moult to adults on 17th February, 1933, after which the following experiments were carried out.

22nd March, 1933. Sheep 35008 injected subcutaneously with an emulsion of five adults.

*Result.*—No reaction.

22nd March, 1933. Sheep 35007 infested on both ears with adults.

*Result.*—No reaction.

26th April, 1933. Sheep 33100 injected subcutaneously with an emulsion of five adults.

*Result.*—No reaction.

26th April, 1933. Sheep 33098 infested on both ears with adults.

*Result.*—No reaction.

Subsequently the four sheep were subjected to an immunity test by the subcutaneous injection of mouse virus. All reacted and one died after showing typical symptoms.

*Conclusion.*—From the above single series of experiments it may not be justifiable to conclude that ticks are incapable of picking up infection while feeding on an immune sheep together with infective ticks. But when it is remembered that the antibody content of the serum of an immune sheep is high, there appears little likelihood of the engorging ticks being able to take up free virus. Consequently it is believed that the immune animal is of no importance as a reservoir of infection.

### DISCUSSION.

The primary object of the series of experiments described was to augment the preliminary report on the transmission of Louping Ill by *Rhipicephalus appendiculatus* (Alexander and Neitz, 1933), by



working out in detail the rôle of the various stages of the tick. The work has shown conclusively that larvae are capable of picking up infection and transmitting it as nymphae, and that nymphae may acquire infection for transmission as adults, but every attempt to demonstrate the passage of virus beyond the adult stage has failed.

Consequently with these investigations an attempt was made to obtain some information upon the tenacity of virus infection in the tick population by determining the effect upon the virus of engorgement upon susceptible and immune sheep. When the work was planned it was intended to carry out supplementary investigations to determine the effect upon the virus of feeding ticks on those unsusceptible species of domestic animals which might normally be encountered on a Louping Ill infected farm. The horse and the bovine were selected in the belief that these species were unsusceptible, and only during the course of the work was this assumption found to be incorrect. It is true that the pathogenesis of the disease in these animals has not been worked out but it has been established that virus may circulate in the peripheral blood of the horse, that a multiplication of virus may take place in the central nervous system of bovines, and that in both species injection of virus is followed by the appearance of virucidal antibodies in the serum to a considerable titre. Consequently there still remains to be worked out the effect upon the virus of tick engorgement on a completely unsusceptible species of animal.

A critical survey of the results obtained illustrates that in work of this nature it is not justifiable to conclude that every tick which ingests infective blood will pick up the infection. Naturally the danger of erroneous conclusions may be reduced to a minimum by feeding large numbers of ticks for each trial. This is a simple matter when larvae and nymphae are being handled, but usually it is impracticable to feed large numbers of adults so that additional significance must be attached to the smaller percentage of positive results obtained with this stage of the invertebrate host.

From a purely academical point of view it is of interest to note that infective nymphae after engorgement on immune sheep, cattle and horses still contain virus at the time of detachment but that this virus in the majority of instances has disappeared by the time the adult stage has been reached, a small percentage of adults only remaining a source of infection. This "cleansing" of the tick cannot be due entirely to the virucidal action of the immune bodies in the blood because a similar phenomenon was encountered in the work on susceptible animals. It is admitted that in the latter cases the results are more difficult to interpret since there always exists the chance that virus might have been circulating before the completion of engorgement, owing to the exceedingly short incubation period of the disease and the comparatively lengthy period of attachment of the ticks. Furthermore, attention must be directed to the low virus content of these adults derived from the nymphae as shown by the failure to produce the disease by subcutaneous injection of tick emulsions, though positive results were obtained by feeding. This activation of the virus has an analogy in Rocky Mountain Spotted Fever (Spencer and Parker, 1929), and Heartwater

(Alexander, 1931), two diseases caused by *Rickettsia*, but as far as the authors are aware it has not been noted in diseases due to filterable viruses. Whether this activation or multiplication represents the completion of a cyclical development of the virus is an interesting problem and an attractive hypothesis for which there is no direct experimental evidence.

From a practical point of view the work indicates that on an infected farm the disease may be controlled and eventually eliminated by running immune animals to serve as hosts for the ticks in order to hasten the development to a succeeding generation always providing that no susceptible animals are introduced during the time to serve as a potential reservoir of infection of this particular vector.

### SUMMARY.

1. Experiments conducted to demonstrate the susceptibility of horses and cattle are described.
2. The appearance of virucidal antibodies in the serum of these animals after infection is demonstrated by a technique of *in vitro* neutralization of virus.
3. The technique of tick feeding investigations is briefly described.
4. It is shown that—
  - (a) larvae of *R. appendiculatus* will pick up infection for transmission as nymphae;
  - (b) nymphae will pick up infection for transmission as adults;
  - (c) the virus does not pass through the egg to the next generation;
  - (d) infective nymphae tend to lose their infection after feeding on immune animals though some of the resulting adults may still be infective;
  - (e) the same occurrence was noted after feeding infective nymphae on susceptible animals but, particularly if sheep are the hosts, there is a danger of the nymphae reacquiring infection before detachment;
  - (f) clean ticks do not acquire infection when feeding on an immune animal simultaneously with infective ticks.
5. The significance of the work is discussed.

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## **The Blesbuck (*Damaliscus Albifrons*) and the Black-wildebeest (*Conochaetes Gnu*) as Carriers of Heartwater.**

By W. O. NEITZ, B.V.Sc., Veterinary Research Officer,  
Onderstepoort.

THE writer (1933) published a preliminary report on the susceptibility of one of the South African antelopes to heartwater. This transmission work has been continued and the details of the experiments are mentioned below.

Besides the blesbuck the black-wildebeest was also found to be susceptible to heartwater.

The antelopes utilized in the transmission work must have been fully susceptible since they were obtained from areas where heartwater is not known to occur, namely the Highveld of the Transvaal and the Orange Free State.

### EXPERIMENT No. 1 (4841).

Blesbuck 34958 was obtained from Theunissen, O.F.S., on the 11th June, 1932.

*Object.*—To attempt transmission of heartwater from a sheep to a blesbuck and to note the reaction.

*Method.*—(a) Virulent heartwater blood was injected subcutaneously and intravenously into the blesbuck.

(b) Blood from this blesbuck was injected intravenously into sheep on the 8th, 12th, and 18th day.

*Result.*—Details of this experiment will be found in Table 1.

The blesbuck did not show any symptoms that could be ascribed to heartwater, but died on the 25th day after inoculation. At autopsy it was found that the marked emaciation was due to a severe panverminosis.

The two sheep injected with blood on the 8th and 12th day did not show any reaction and following a subsequent immunity test both died from typical heartwater.

The third sheep injected on the 18th day reacted to heartwater and died.

TABLE I.

D.O.B. No. of animal.	Injected from.	Date.	Interval in days after injection of blesbuck.	Injected blood.	Result.
Blesbuck 34958...	Sh. 34407	28/10/32	—	10 c.c. s.c. 5 c.c. i.v.	Died on the 25th day after injection as result of panvermiosis. No symptoms of heartwater seen.
Sh. 34761.....	Bb. 34958	4/11/32	8	10 c.c. i.v.	Did not react. On immunity test animal died from heartwater.
Sh. 31580.....	Bb. 34958	8/11/32	12	10 c.c. i.v.	Did not react. On immunity test animal died from heartwater.
Sh. 34233.....	Bb. 34958	14/11/32	18	10 c.c. i.v.	Reacted to heartwater on the 8th day and died on the 15th day. Rickettsia ruminantium could be demonstrated in brain section. Subinoculation from this sheep into two susceptible ones further confirmed diagnosis of heartwater.

TABLE II.

D.O.B. No. of animal.	Injected from.	Date.	Interval in days after injection of blesbuck.	Injected blood.	Result.
Blesbuck 34961...	Sh. 35003	6/12/32	—	10 c.c. s.c.	No symptoms of heartwater seen.
Sh. 35033.....	Sh. 35019	6/12/32	0	5 c.c. i.v.	No reaction. On immunity test animal died from heartwater.
Sh. 34212.....	Bb. 34961	17/12/32	11	5 c.c. i.v.	" " " "
Sh. 35023.....	"	22/12/32	16	10 c.c. i.v.	" " " "
Sh. 35027.....	"	28/12/32	16	"	Reacted to heartwater on the 16th day and died.
Sh. 34090.....	"	31/12/32	22	"	No reaction. On immunity test animal died from heartwater.
Sh. 35541.....	"	31/12/32	25	"	" " " "
Sh. 35821.....	"	4/1/33	25	"	" " " "
Sh. 35829.....	"	"	29	"	" " " "
Sh. 35035.....	"	"	29	"	" " " "
Sh. 34792.....	"	"	"	"	Died on the 23rd day after injection. Cause of death not known. Subinoculation from this animal into susceptible heartwater sheep produced no heartwater reaction.
Sh. 35807.....	"	16/1/33	41	"	No reaction. On immunity test animal died from heartwater.
Sh. 35825.....	"	"	41	"	" " " "

## EXPERIMENT No. 2.

Blesbuck 34961 was obtained from Theunissen, O.F.S., on the 11th June, 1932.

*Object.*—To repeat the transmission of heartwater from sheep to a blesbuck and to note the reaction.

*Method.*—(a) Blood from this blesbuck was injected into a susceptible heartwater sheep in order to see whether this animal harboured any other disease that could be transmitted by blood subinoculation.

(b) Blood from three sheep reacting to heartwater was pooled and injected subcutaneously and intravenously into the blesbuck.

(c) Blood from this blesbuck was injected intravenously into sheep on the 11th, 16th, 22nd, 25th, 29th and 41st days.

*Result.*—Details of this experiment are given in Table II.

(a) The blesbuck did not harbour any disease that could be transmitted by blood subinoculation.

(b) The blesbuck did not react to heartwater and remained healthy.

(c) One of the sheep 35027 injected on the 16th day reacted to heartwater on the 16th and died on the 24th day after injection. None of the other sheep developed heartwater and when the immunity of these sheep was tested they all died from heartwater. One of the sheep 34792 injected on the 29th day died from causes other than heartwater, and blood from this animal when injected into two susceptible heartwater sheep did not produce a reaction.

## EXPERIMENT No. 3.

Blesbuck 33606 was obtained from Standerton, Transvaal, on 16th March, 1932, and splenectomized by Dr. Quinlan of this Institute on the 12th April, 1932. This animal was found to be fully susceptible to *Anaplasma marginale*. After recovery from this infection it was utilized for the heartwater work.

*Object.*—To attempt transmission of heartwater from sheep to a splenectomized blesbuck and to note the reaction.

*Method.*—(a) Blood from this blesbuck was injected into two susceptible heartwater sheep in order to see whether this animal harboured any other disease that could be transmitted by blood subinoculation.

(b) Blood from two sheep reacting to heartwater was injected subcutaneously and intravenously into the blesbuck.

(c) Blood from this blesbuck was injected intravenously into susceptible heartwater sheep on the 12th, 15th and 20th day.

*Result.*—Details of the subinoculations will be found in Table III.

(a) The two sheep injected did not react.

TABLE III.

D.O.B. No. of animal.	Injected from.	Date.	Interval in days after injection of blesbuck.	Injected blood.	Result.
Sh. 35022.....	Bb. 33606	24/1 '33	--	10 c.c. i.v.	No reaction observed.
Sh. 35032.....	Sh. 34177	26 1 '33	--	20 c.c. i.v.	No reaction observed.
Blesbuck 33606..	Sh. 35033			10 c.c. i.v.	Died on the 21st day after injection, showing heartwater lesions at autopsy. Rickettsia ruminantium could be demonstrated in the intima smears from the jugular vein.
Sh. 34425.....	Bb. 33606	7 2 33	12	10 c.c. i.v.	Reacted to heartwater on the 10th day and died on the 16th day.
Sh. 35025.....	"	"	12	" "	Reacted to heartwater on the 10th day and died on the 25th day.
Sh. 34329.....	"	10-2 33	15	10 c.c. i.v.	Reacted to heartwater on the 9th day and died on the 14th day.
Sh. 34994.....	"	"	15	" "	Reacted to heartwater on the 9th day and died on the 12th day.
Sh. 24078.....	"	15-2 33	20	" "	Reacted to heartwater on the 7th day and died on the 10th day.
Sh. 35811.....	"	"	20	" "	Reacted to heartwater on the 6th day and died on the 12th day.

TABLE IV.

D.O.B. No. of animal.	Injected from.	Date.	Interval in days after injection of black-wildebeest.	Injected blood.	Reaction.
5193 Black-wilde-beest....	37778	22-8-33	--	10 c.c. i.v.	No reaction observed.
	37272			20 c.c. s.c.	
	37385	28-8-33	6	10 c.c. i.v.	Did not react to heartwater.
Sh. 37532.....	"	"	6	" "	Did not react to heartwater.
Sh. 37698.....	"	4-9-33	13	" "	Did not react to heartwater.
Sh. 37837.....	"	"	13	" "	Reacted to heartwater on the 18th day, and died on the 20th day.
Sh. 38000.....	"	"	23	" "	Reacted to heartwater on the 9th day and died on the 13th day.
Sh. 37805.....	"	14 9 33	23	" "	Reacted to heartwater on the 13th day and died on the 17th day.
Sh. 37995.....	"	"	23	" "	Intima smears from the jugular vein showed numerous Rickettsia ruminantium.
Sh. 36937.....	"	21 9 33	30	" "	Did not react to heartwater.
Sh. 37986.....	"	"	30	" "	Reacted to heartwater on the 12th day and died on the 15th day.
Sh. 34988.....	"	5/10-33	44	" "	Rickettsia ruminantium could be demonstrated in sections prepared from the hippocampus.
Sh. 35009.....	"	"	44	" "	Did not react to heartwater.

(b) The blesbuck died on the 21st day after injection, showing heartwater lesions at autopsy. *Rickettsia ruminantium* could be demonstrated in the intima smears prepared from the jugular veins.

(c) All the sheep subinoculated on the 12th, 15th and 20th day reacted to heartwater and died.

#### EXPERIMENT No. 4 (5126).

Black-wildebeest 5193 was obtained from the farm Spesbona, Geneva Station, Orange Free State, and arrived at Onderstepoort on 25th June, 1932.

*Object.*—To attempt transmission of heartwater from sheep to a black-wildebeest and to note the reaction.

*Method.*—(a) Blood from three sheep reacting to heartwater was pooled, and injected subcutaneously and intravenously into the black-wildebeest.

(b) Blood from this black-wildebeest was injected intravenously into susceptible heartwater sheep on the 6th, 13th, 23rd, 30th and 44th day.

*Result.*—Details of this experiment will be found in Table IV.

(a) The black-wildebeest did not show any symptoms and remained healthy.

(b) No reactions were produced in the sheep inoculated on the 6th and 44th days. Of the two sheep inoculated on the 13th day one reacted and died, of the two sheep inoculated on the 23rd day both reacted and died, and of the two injected on the 30th day one reacted and died from heartwater.

#### CONCLUSIONS.

(1) The transmission of heartwater to three blesbuck and a black-wildebeest is discussed.

(2) The virulence of the heartwater "virus" did not change by passage through the antelopes.

(3) The antelopes did not show any clinical symptoms that could be ascribed to heartwater. In case of the splenectomized blesbuck, however, heartwater lesions were observed at autopsy, and *Rickettsia ruminantium* was demonstrated in the intima smears prepared from the jugular vein.

(4) Heartwater "virus" could be demonstrated by blood subinoculations into susceptible sheep on the 16th and 18th day in two of the blesbuck, from the 12th to the 20th day in the splenectomized blesbuck and from the 13th to the 30th day in case of the black-wildebeest.



(5) The fact that heartwater "virus" could be demonstrated in the blood of one animal for 9 days and in another for 18 days gives one good reason to believe that antelopes can act as reservoirs for heartwater and that the possibility exists for ticks to infect themselves by feeding on these animals.

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## Section III.

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# Parasitology.

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- ORTLEPP, R. J.      On some helminths from the "Nylghiae" - *Boselaphus tragocamelus* (Pall) with observations on the parasitic larval stages of the stomach worm *Ashworthius martinaglia* sp. n.
- ORTLEPP, R. J.      On the metacecaria and adult of *Glinostomum van der horsti* sp. n. A nematode parasite of fishes and herons.



## On Some Helminths from the "Nylghiae"— *Boselaphus tragocamelus* (Pall) with Obser- vations on the Parasitic Larval Stages of the Stomach Worm *Ashworthius martinagliai* sp. n.

By R. J. ORTLEPP, M.A., PH.D., Research Officer,  
Onderstepoort.

Through the kindness of Dr. G. Martinaglia, Veterinarian to the Municipality of Johannesburg, two nematode and two trematode species collected from the "Nylghiae" were placed at the disposal of the writer for study. The host was a very recent importation from India and had to be destroyed soon after arrival at the Johannesburg Zoological Gardens because of injuries it had received in transit. The writer wishes to express his appreciation to the donor for having placed this interesting material at his disposal.

Class: **NEMATODA** Rudolphi, 1808.

Superfam.: STRONGYLOIDEA Weinland, 1858.

Fam.: TRICHOSTRONGYLIDAE Leiper, 1908.

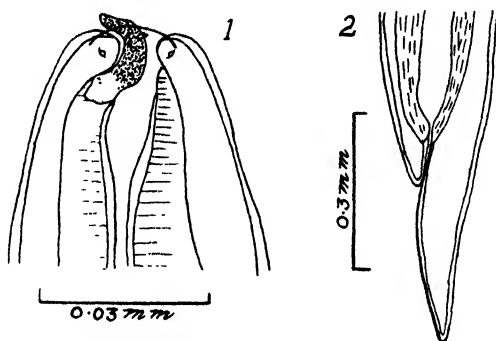
Sub.-fam.: TRICHOSTRONGYLINAE Leiper, 1908.

*ASHWORTHIUS MARTINAGLIAE* sp. n.

Numerous specimens of this species were collected from the abomasum of the Nylghiae. They were in a much shrunken condition, but fortunately they straightened out and appeared to have resumed their normal shape after immersion in lacto-phenol for a day. Superficially they closely resemble the common stomach worm of the sheep (*Haemonchus contortus*), the females showing the characteristic barber-pole markings of this species.

The body is slender in both sexes and is attenuated towards the anterior extremity; this attenuation is most marked from about the level of the cervical papillae to the anterior end, which in the mature males has a diameter of only 0.025 to 0.026 m.m., and in the mature females of 0.027 to 0.028 m.m. The whole body shows very fine cuticular annulations 0.0015 to 0.002 m.m. apart. The cervical papillae are very prominent, laterally placed, 0.26 to 0.33 m.m. from the anterior end. The excretory pore is found in a ventral transverse groove and is situated from 0.22 to 0.27 m.m. from the anterior end.

The mouth (Fig. 1) is bounded by four inconspicuous lips of which the dorsal and the ventral are small. The six circum-oral papillae are all carried by the lateral lips, the two laterals being placed slightly anterior of the sub-ventrals and sub-dorsals. The buccal cavity is small and is not tilted dorsally as in the genotype. There is a conspicuous buccal lancet arising from the dorsal segment of the oesophagus and bent dorsally after emerging from the mouth. Its roots are similar to those of the genotype, triangular in a dorsal and somewhat rectangular in a lateral view.

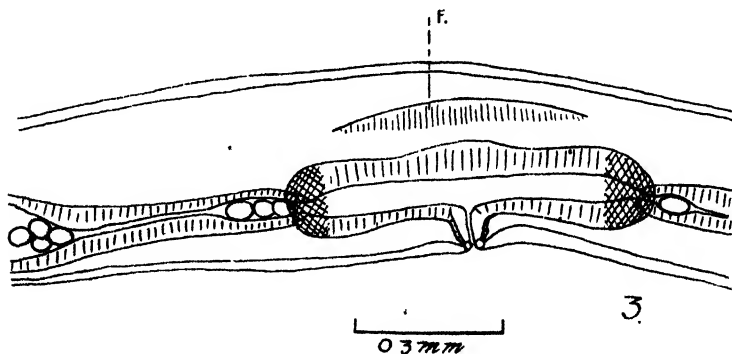


*Ashworthius martinaglii* sp. n.

Fig. 1.—Anterior extremity, lateral view.

Fig. 2.—Posterior extremity of female.

The oesophagus is long and claviform, increasing in diameter from 0.022 m.m. at the anterior end to 0.14 m.m. at its posterior end; in the females it varies in length from 1.77 to 1.8 m.m., in the males from 1.49 to 1.52 m.m.; it is encircled by the nerve ring just anterior to the level of the excretory pore.

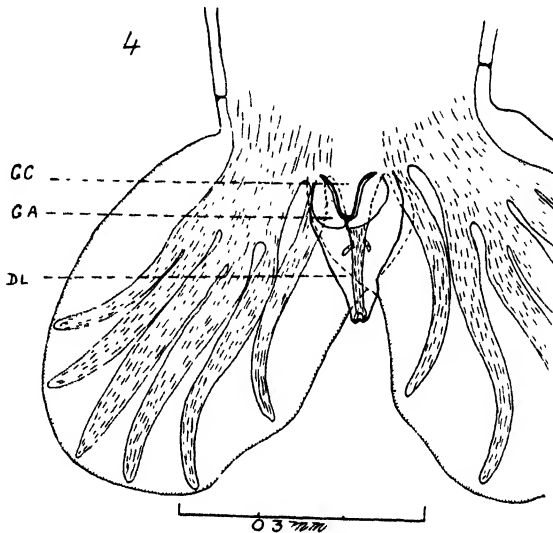


*Ashworthius martinaglii* sp. n.

Fig. 3.—Vulva and associated organs, showing cuticular flange F.

*Female*.—Adult specimens, containing eggs, vary in length from 17.5 to 19 m.m. with a maximum thickness just anterior of the vulva of 0.37 to 0.4 m.m. The body tapers towards both extremities and terminates posteriorly in a pointed tail 0.28 to 0.34 m.m. long (Fig. 2) with two conspicuous caudal papillae about 0.1 m.m. from its tip.

The vulva is a small transverse slit situated in the posterior half of the body at about the junction of the fourth and last fifth; in most cases it is only very slightly protuberant. It is encircled by a cuticular ring lying just below the cuticular body covering. A linguiform process is entirely absent, but lateral of the vulva there may be one or two small ring-like expansions of the cuticle (Fig. 3); in twelve specimens six had this ring on the left side only, three on the right side only, two on both sides, and one had none. Adjacent to the vulva there is a small papilla limited to the *right* side only. The vagina is short and transverse and leads directly into the straight ojectors measuring from 0.7 to 0.9 m.m. inclusive of the sphincters. The ovaries are wound round the intestines as in *Haemonchus contortus*. The eggs are oval and thin-shelled and are segmented *in utero*; they vary in size from 0.07 to 0.073 m.m. long by 0.038 to 0.044 m.m. broad.

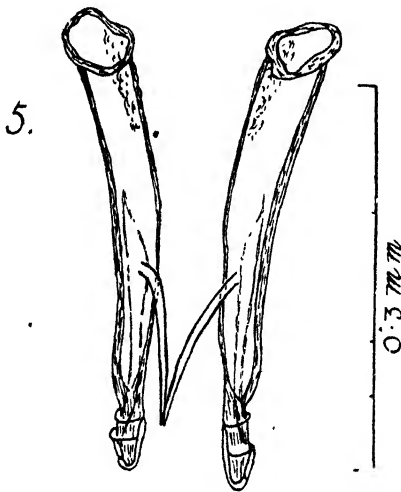


*Ashworthius martingali* sp. n.

Fig. 4.—Ventral view of bursa. GC=genital cone; GA=genital appendage; DL=dorsal lobe.

*Male*.—Fully grown males vary in length from 11 to 13 m.m. with a maximum thickness just anterior of the bursa 0.23 to 0.26 m.m. The bursa is ample and consists of two elongate lateral lobes with finely serrated edges, which overlap each other at their bases on the dorsal side (Fig. 4). The dorsal lobe is relatively small and symmetrical and lies ventral to the overlapping portions of the lateral lobes; it is somewhat heart-shaped and carries only the dorsal ray, the externo-dorsals being lodged in the lateral lobes; its tip is slightly notched and its edges are not serrated. The ventral and lateral rays have a common stout stem, which sub-divides into three branches: a ventral, median, and dorsal. The ventral branch divides to form the two ventral rays, of which the ventro-ventral is smaller; it runs adjacent to the latero-ventral for about half its length, after which it is slightly bent towards the ventral side; only the tip of the latero-ventral is bent ventrally. The median branch gives rise to

externo-lateral and medio-lateral rays; the former is the largest of the bursal rays and passes straight to the edge of the bursa; the medio-lateral runs parallel to it for about two-thirds of its length, after which it becomes slightly arched dorsalwards. The dorsal branch forms the postero-lateral ray only; its proximal half lies more or less parallel to the postero-lateral rays, but its distal half is more strongly arched dorsalwards. The externo-dorsal ray takes its origin from the base of the dorsal ray before it enters into the dorsal lobe; it is slightly arched and rib-shaped. The dorsal ray is straight and has a stout base; it tapers towards its distal extremity, which is divided into two short branches; at about its middle it gives off two short branches on its ventral side; these bend ventralwards and terminate in the ventral surface of the dorsal lobe. The spicules are stout and dark brown in colour (Fig. 5). Their dimensions are 0.315 to 0.372 m.m. long by 0.044 to 0.048 m.m. broad at their proximal ends. Each spicule carries two transverse ridges towards its tip; these are limited to its dorsal and outer faces, and when viewed from either the dorsal or ventral aspect the spicule appears to carry two barbs; these are situated some 0.038 and 0.06 m.m. respectively from the somewhat blunt spicular tip. At about the junction of its third and fourth-fifths each spicule gives rise to a spike which arises from the dorsal surface of the spicule, somewhat more towards its inner face; it passes backwards along the dorsal side of the spicule to about the level of the proximal "barb-like" ridge.



*Ashworthius martinagliai* sp. n.

Fig. 5.—Ventral view of spicules after removal from body.

On the ventral surface each spicule carries two longitudinal ridges, which converge and meet proximally, but terminate distally each in one of the barb-like ridges.

A gubernaculum is absent; but a delicate prebursal papilla is lodged on either side just anterior to the base of the bursa.

There is a well-developed genital cone 0.062 to 0.078 m.m. long, somewhat pyramidal in shape, and flanked on its lateral side by two conspicuous genital appendages, which are slightly curved over its distal end.

The genus *Ashworthius* was created by le Roux (1930) for the reception of a stomach worm *A. partoni*, le Roux (1930) from the bushbuck (*Tragelaphus sylvaticus*), Zululand. The species described above agrees with the genotype in that it carries a buccal tooth, has a symmetrical dorsal bursal lobe and has no gubernaculum in the male or linguiform process in the female. It differs from it, however, in its much smaller head, entire separation of the dorsal bursal lobe from the lateral lobes, differently shaped spicules, larger ventro-ventral rays, presence of a papilla on the right side of the vulva and usual presence of one or two cuticular flanges on the body at the sides of the vulva. These differences may, with the increase of our knowledge of the genotype, perhaps warrant the creation of a separate genus for its reception; unfortunately, the material of the genotype is at present too meagre to allow of a more detailed comparison.

In 1922 Baylis and Daubney described *Hacmonchus cervinus* from the spotted deer (*Cervus axis*), India. Unfortunately their material was in a poor condition, and the only male present had an incomplete bursa, which was thus not described; this species agrees with that described above in its very small head (0.023 to 0.025 m.m.), and in the absence of a linguiform process in the female; it differs from it, however, in its smaller size (13-15 m.m.), its longer female tail (0.37 m.m.), absence of a papilla on the right side of the vulva and absence of cuticular flanges lateral of the vulva.

### *Larval Stages.*

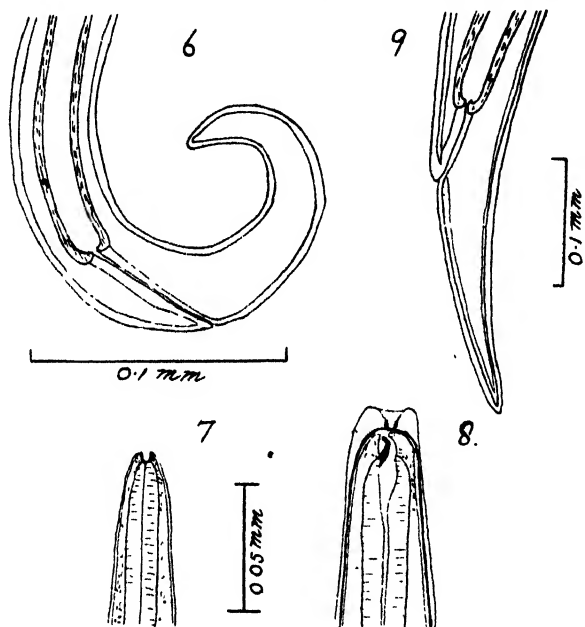
Among the adult specimens of this parasite there were several examples of early fourth-stage larval stages and a few examples of the final female fourth stage. The early fourth-stage larvae are small and slender and vary in length from 2.23 m.m. to 2.76 m.m., with a maximum thickness of 0.044 to 0.047 m.m. The cuticle carries very fine annulations, which extend from just behind the oral aperture up to almost the top of the tail. The body is straight and of a more or less uniform thickness, except for the two extremities which are tapered; the tail (Fig. 6) is pointed and is twisted dorsalwards and varies in length from 0.09 to 0.11 m.m. There are two distinct and peg-like cervical papillae lodged from 0.15 to 0.18 m.m. from the anterior extremity.

The larval buccal capsule is small, but quite distinct, and is somewhat bowl-shaped, its depth being equal to its breadth (Fig. 7), being about 0.005 m.m. in both dimensions. It leads into a simple club-shaped oesophagus, 0.37 to 0.42 m.m. long, which is about 0.01 broad at its anterior and 0.029 m.m. broad at its posterior end. It is encircled by the nerve ring just anterior to the level of the cervical papillae, and the excretory pore is found about midway between the levels of these two structures.



The genitalia are still undifferentiated and still consist of a lens-shaped group of cells situated ventral of the intestine at about the junction of the third and fourth body fifths.

Two female specimens representing the final fourth stage were present. Both are ensheathed, the one showing the provisional buccal capsule only, while the other already shows the final mouth with its buccal lancet (Fig. 8). The former is 4.57 m.m. long, with a maximum thickness of 0.1 m.m., and the tail is pointed and 0.16 m.m. long (Fig. 9). These three measurements for the other larvae are 4.3 m.m., 0.1 m.m., and 0.19 m.m. respectively. The position of the future vulva is indicated by a ventral swelling 0.94 and 0.97 m.m. from the tip of the tail in the two larvae respectively; the vagina, however, has not yet acquired its external opening. In both



*Ashicorthis martinaghai* sp. n. .

Fig. 6.--Tail of early fourth-stage larva.

Fig. 7.--Cephalic extremity of early fourth-stage larva.

Fig. 8.--Cephalic extremity of ensheathed fourth-stage larva.

Fig. 9.--Caudal extremity of ensheathed fourth-stage female larva.

worms the development of the internal genitalia is at about the same stage and consists of a short transverse vagina leading into an unpaired hollow and longitudinal portion, which at both ends joins on to the ovejectors; these in turn are followed by a short uterine portion which finally join on the short ovarian portion, which at this stage consists of a simple rod of single cells.

The material unfortunately contained no fourth-stage males. The smallest fifth-stage male is a single specimen 4.5 m.m. long and 0.12 m.m. broad just anterior of its bursa. It shows all the characteristics of the adult, except that the lateral bursal lobes are relatively much shorter, and in consequence the unpaired dorsal lobe is of the same length as the lateral lobes.

Superfam.: TRICHUROIDEA Railliet, 1916.

Fam.: TRICHURIDAE Raill., 1915.

Sub-fam.: TRICHURINAE Ransom, 1911.

TRICHURIS GLOBULOSUS (v. Linst., 1901).

Six specimens (three males and three females) of this species were obtained from the caecum. They agree in all essentials with Sprehn's redescription of this species, except with regard to the breadth of the spicule. Sprehn's gives the spicule as 0.08-0.09 m.m. broad. In my specimens the spicule is 0.038-0.04 m.m. broad. Representative material, from sheep, in this laboratory has spicules varying in breadth from 0.038 to 0.044 m.m. These measurements are more in agreement with those given by Baylis (1932) (0.0325-0.05 m.m.) and Gebauer (0.032 m.m.).

### Class TREMATODA.

Super-fam.: PARAMPHISTOMOIDEA Stiles and Goldberger, 1910.

Fam.: PARAMPHISTOMIDAE Fiscoeder, 1901.

Sub-fam.: PARAMPHISTOMINAE Fiscoeder, 1901.

COTYLOPHORON COTYLOPHORON (Fisch, 1901) Stiles and Goldb., 1910.

This species was represented by ten specimens collected, with the following species, from the Rumen.

Fam.: GASTROTHYLACIDAE Stiles and Goldberger, 1910.

Sub-fam.: GASTROTHYLACINAE Stiles and Goldberger, 1910.

GASTROTHYLAX CRUMENIFER (Crepin, 1847),  
Poirier, 1883.

About two dozen specimens were present, varying in length from 6 to 10 m.m.

### RÉSUMÉ.

Four helminths are recorded from the Nylghiae (*Bosclaphus tragocamelus*), India. One of these—*Ashworthius martinagliai*—a trichostrongylid stomach-worm, is new to science. Its morphology is described, together with the early fourth stage and late fourth stage female larva. *Trichuris globulosus*, *Cotylophoron cotylophoron* and *Gastrothylax crumenifer* are recorded from this host.

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SOME HELMINTHS FROM THE " NYLGHIAE ".

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ADDENDUM.

Since writing the above the writer has seen an article by SCHULZ (1933)\* wherein he describes *Ashworthius sidemi* sp.n. from *Pseudaris hortulorum*. This species differs from the writer's principally by the shape of its spicules, the origin of its ventro-ventral bursal ray, and the presence of a linguiform process in front of the vulva.

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\* SCHULZ, R. ED. (1933). *Ashworthius sidemi* n.sp. (Nematoda, Trichostrongylidae) aus einem Hirsch (*Pseudaris hortulorum*) des fernen Ostens. *Zeit. Parasitk.*, Vol. 5, Nos. 3/4, pp. 735-739, Berlin.

## On the Metacercaria and Adult of *Clinostomum van der horsti* sp. n., a Trematode Parasite of Fishes and Herons.

By R. J. ORTLEPP, M.A., Ph.D., Research Officer, Onderstepoort,  
South Africa.

A PRESERVED specimen of the fish *Gnathonemus macrolepidotus* was kindly placed at the disposal of the writer by Prof. C. J. van der Horst of the Zoological Department, University of the Witwatersrand. This fish had died in the aquarium of the University, and on being opened Prof. v. d. Horst noticed that it was heavily parasitized by a larval trematode. The writer identified these as the metacercariae of a clinostome, and arrangements were then made to obtain fresh fish material from Prof. v. d. Horst in order that the fresh metacercariae could be fed to a suitable host in order to obtain the adult parasite. Two live fishes were obtained, both of which proved on dissection to be heavily parasitized. These were fed to a young blackheaded Heron—*Ardea melanocephala*—which had just left the nest, and which on examination had been found to be free of helminths. The writer wishes to express his sincere thanks to Prof. v.d. Horst for providing him with this material and to Dr. Bigalke of the National Zoological Gardens, Pretoria, for placing the heron at his disposal.

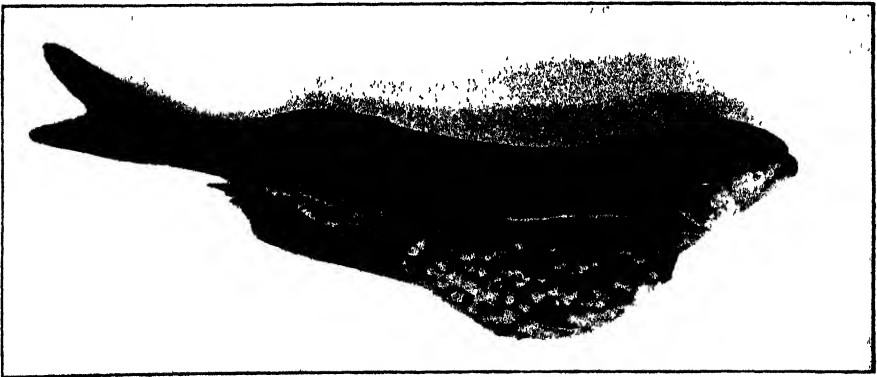


Fig. 1.—*Gnathonemus macrolepidotus* (natural size) with Metacercariae of *Clinostomum van der horsti* sp. n. in situ.

*Metacercaria*.—The parasitized fishes which were examined were each found to contain about 50 metacercariae; these for the most part filled up the whole of the body cavity, being attached to the lining of the body cavity (Fig. 1). None were attached to the internal organs.

and only a few were found to be superficially buried in the muscles of the abdominal body wall. Each metacercaria was somewhat rounded, about 2 to 3 mm. in diameter, and surrounded by a thin transparent envelope; they were all lying close against each other so that collectively they looked somewhat like a bunch of grapes.

On removing the envelope it was found that the contained metacercaria was folded on itself dorsally, and when placed in normal saline it showed active movements of elongation and contraction. Some of the metacercariae were fixed according to Looss' method in Schaudin's solution, while others were examined alive under slight pressure. Serial longitudinal sections were cut of the former. As the morphology of the metacercariae is practically identical with that of the adult, it will be discussed later on. The only differences are that the metacercariae are slightly smaller, the preserved specimens being from 5 to 6 mm. long; the vitellaria have not yet made their appearance; and the genital glands are smaller.

One of the fishes containing the living metacercariae was fed to the heron by forcibly sliding the fish down the gullet. As the heron was kept in an enclosed cage there was no likelihood that it could contract an infection other than from the infected fish. It had free access to a bowl of clean tap water, and its daily food consisted of chopped up beef and mutton. The heron was unfortunately not examined until the 8th day after infection, on which date mature worms were already present; it is thus not possible to state when the adults first took up their final location. Yamaguti (1933) found the adults of *C. complanatum* (Rud) 45 hours after feeding the metacercariae to a heron. Nine of these adults were seen firmly attached to the mucous membrane of the mouth round about the glottis, and one was fixed inside the glottis. An examination of the upper half of the oesophagus revealed no further worms; as the heron had swallowed about 50 metacercariae, the proportion which attained maturity was surprisingly low.

The worms were firmly attached by both their oral and ventral suckers and it required considerable force to remove them. The ventral sucker draws a portion of the mucous membrane into its lumen and, using this attachment to anchor itself, the worm bends its head end sharply ventralwards and bores into the mucous surface with its oral extremity. There is a muscular ridge or collar just behind the mouth, and this, when pressed against the adjacent tissues, firmly fixed the head in the tissues. On being pulled the ventral anchor is the first to relinquish its hold, and a further pull frees the head which comes away with a portion of the surrounding tissues. A deep scar is left which tends to bleed profusely. During the course of the next few weeks the position of the worms was noted every day, and it was found that they change their position fairly often; the old place of fixation was always represented by a scar.

The adult worms are bright pink in colour, whereas the metacercariae are of a creamy colour. The pink colour is due to the blood on which the adults feed. As free blood was seen to be present in the vicinity of the worms, and as the head end is so firmly attached that there is little likelihood of blood escaping round the sides of the head,

it is probable that blood is pumped through the gut to be expelled at the "anus" in somewhat the same way as that described for *Ancylostomum caninum* by Wells (1931); at any rate the worms perform considerable contractile movements *in situ*, so that it would be quite easy for the blood to pass to the exterior before it had time to become digested.

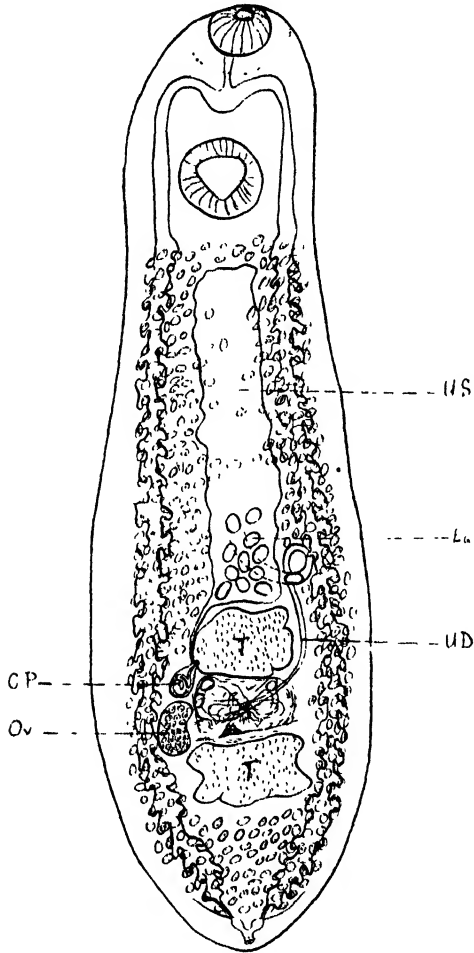


Fig. 2.—Ventral view of entire worm, drawn from living specimen slightly flattened. C.P.=cirrus pouch; Eg=eggs; Ov.=ovary; U.D.=uteroduct; U.S.=uterine sac.

*Morphology.*—When fixed in Schaudin's solution the metacercariae and the adults have much the same shape, except that the former is slightly smaller, being from 5 to 6 mm. long whereas the adults are from 6.25 to 7 mm. in length. The body (Fig. 2) is broadest in the region of the genital glands where it is 2 mm. broad and 0.9 mm. thick in the adult. Behind this region the body tapers rather quickly to end in a bluntly pointed tip. Anteriorly the body narrows to the level of the ventral sucker, after which the breadth remains more or less uniform to become suddenly rounded off at the

oral extremity. In transverse section the body is somewhat semi-circular, having a somewhat flattened or slightly concave ventral surface and a convex dorsal surface. The cuticle is provided with very minute spines, about 0.01 mm. long; these are limited to the postacetabular region of the body and their tips hardly pierce the cuticular surface. Even in sections they are rather difficult to make out. They are present in both the metacercaria and adult.

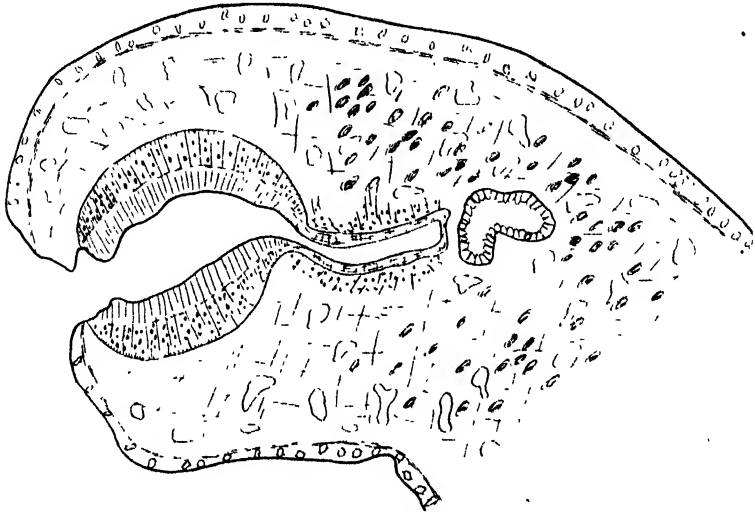


Fig. 3. -Vertical section of oral extremity showing constitution of wall of oral sucker and absence of pharynx.

The oral sucker is terminal, and its aperture lies in the centre of the somewhat flattened anterior surface; it has a slightly oblong shape, being 0.42 mm. long and 0.39 mm. broad (Fig. 3). Its wall is divisible into three parts, namely an inner purely muscular portion with radial fibres; a middle portion consisting of radial muscle fibres and glandular cells, and a thin outer portion consisting of radial muscle fibres only. A very thin layer of longitudinal muscle fibres separates the inner from the middle portion.

The ventral sucker is very muscular and is situated about 1 mm. behind the anterior extremity, in preserved specimens it is somewhat sunken into the body and its inner edge is almost adjacent to the dorsal body wall. It is about 0.9 mm. broad by 0.78 mm. long and has a depth of 0.7 mm. In living specimens its external opening is somewhat triangular with the apex pointing backwards. Its wall is very muscular 0.45 mm. thick and consists of radially arrayed muscle fibres, between which isolated rows of small nuclei are seen.

The cuticular layer varies in thickness from 0.06 mm. (metacercaria) to 0.09 in the adult. Immediately underneath it there is a very thin layer of longitudinal muscle fibres, followed by a zone of circular fibres from 0.025 to 0.047 mm. in thickness; then there is an inner layer of longitudinal fibres 0.013 to 0.022 mm. in thickness which is then followed by an inner layer of circular fibres, this layer varying in thickness from 0.032 to 0.06 mm. As pointed out

by Baer (1923) the presence of all these muscle layer in the sub-cuticular section is very reminiscent of that which is seen in cestodes. The parenchyma is also very well supplied with muscle fibres, circular, longitudinal and vertical, and in among these, especially on the preacetabular region, there are numerous glandular cells.

*Digestive System.*—The oesophagus is short and is composed of a weak muscular layer consisting of a mixture of radial and longitudinal muscle fibres; it is surrounded by a large number of glandular cells which appear to be similar to those found in the wall of the oral sucker. A pharynx is entirely absent. The two intestinal branches extend almost to the posterior extremity; from behind the level of the acetabulum each gives rise on its outer and inner surfaces to a number of conspicuous sacculations. A very small canal passes backwards from the distal end of each intestinal caecum to open into the small excretory vesicle which in its turn opens to the exterior in a fairly conspicuous pore situated subterminally and ventral (Fig. 4). That the connections between the caeca and excretory vesicle have a functional significance was noted in all the living specimens

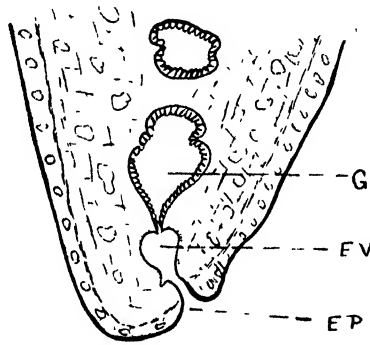


Fig. 4. Reconstruction from four serial sections showing connection between intestinal caeca (G) and excretory vesicle (E.V.). E.P.=excretory pore.

examined, metacercariae and adults. The intestinal contents naturally flowed down these canals and passed to the exterior via the excretory pore; this could also be very easily induced when the worms were placed under coverslips under slight pressure just sufficient to make them transparent under the microscope. These connections between the caeca and excretory vesicle were also shown in section of worms which had been killed and fixed without previous examination under the microscope. The material excreted in the adult specimens consisted of blood cells in various stages of digestion.

These communications between the digestive system and excretory vesicle have recently been described and figured by Yamaguti (1933) for *Clinostomum complanatum* (Rud., 1819); although he saw in living specimens that the intestinal contents are sometimes forced into the vesicle he does not think it probable that a normal functional anus is therefore present. However, the writer, after an examination of living specimens hold a contrary view as stated above.



*Excretory System.*—This system consists of many branched tubules lying in the parenchyma and sub-cuticular tissues; these collect into larger tubes which pass backwards driving the excretory material backwards; one of these lies just outside of the intestinal caeca and its lumen is provided with bunches of cilia which lash backwards; these tubes join to transverse tubes just anterior of the excretory vesicle and these in turn open into two large wavy tubes, one on either side between the body margin and the longitudinal tubule described above; these large canals disappear at about the level of the ventral sucker. Except for its posterior fifth its lumen is free of cilia; these large tubes open each into one of the horns of the V-shaped excretory vesicle. The limbs of the excretory vesicle are slightly swollen and in section have a greater diameter than that portion which opens to the exterior. The excretory pore lies in the mid ventral line only very slightly removed from the posterior tip of the body.

*Reproductive System.*—The genital glands are situated in the posterior quarter and consists of two slightly lobed testes, between which and slightly to the right is the smaller and somewhat spherical or crescentic ovary (Fig. 1). The testes are more or less of equal size, the anterior, however, may appear smaller; they fill up most of the space between the intestinal caeca; they are about 1 mm. broad by 0.7 mm. long and 0.5 mm. thick and are about 0.4 mm. apart. The cirrus pouch is somewhat pyriform in shape, and lies just posterior of the anterior testes on its right side; it opens into the genital atrium at the level of the anterior testes. It is muscular and is about 0.6 mm. long and 0.34 to 0.37 mm. broad at its base; the cirrus is very muscular and voluminous and its surface is studded with cuticular bosses similar to those described for *C. lophophallum* Baer, 1933. A very large *vesicula seminalis* is present in the base of the cirrus pouch; it is very much coiled on itself. A *pars prostatica* is absent. The genital atrium opens on the ventral surface a little to the right of the median body line and at about the level of the middle of the anterior testes; it receives the openings of the cirrus sac and metraterm on its posterior and anterior faces respectively (Fig. 5).

The ovary is situated on the right side between the testes; in ventral view it is slightly crescentic in shape, but is somewhat spherical in dorso-ventral section. It is about 0.32 mm. long, 0.28 mm. thick and 0.25 mm. broad (Figs. 1 and 6A). The oviduct is short and is lined with columnar cells bent away from the ovary; antero-dorsally it joins with the Laurer's Canal, which is relatively very muscular and long and may become enlarged just before entering the oviduct; the external opening of the Laurer's Canal can very easily be seen in the dorsal midline in the living specimen when examined under moderate magnification. The yolk reservoir opens into the oviduct on its posterior face soon after it has joined Laurer's Canal; it is a triangular organ, the two points of its base being continued into the transverse vitelline ducts. The oviduct now continues as the ootype and further as the uteroduct which performs several convolutions on itself between the testes and ovary to eventually pass forwards on the left side of the anterior testes and after being coiled once on itself it enters into the uterine sac on its

dorsal surface about 0.8 mm. anterior of the anterior testes. The shell gland is very poorly developed and consists of relatively few cells. The uterine sac extends from the anterior testis to about 0.4 mm. posterior of the acetabulum and contains numerous eggs. The metratrum is a straight canal passing backwards and ventralwards from the right posterior corner of the uterine sac to open on the anterior face of the genital cirrus. The distal portion of the yolk reservoir duct, the ootype and the first portion of the uteroduct are lined by cilia which perform a lashing movement towards the uteroduct.

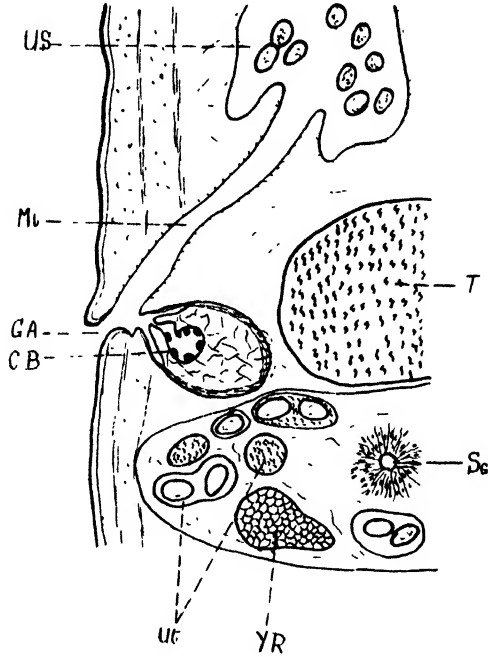


Fig. 5.- Longitudinal section through genital atrium (G.A.). C.B. - cuticular bosses on cirrus. Mt - metratrum. Sg - shell gland. Ut. - sections of uteroduct. U.S. - uterine sac. Y.R. - yolk reservoir.

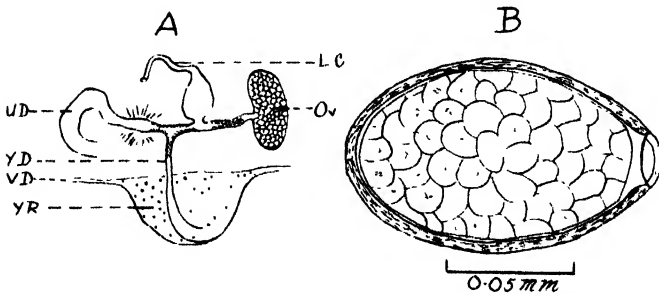


Fig. 6.—A. Reconstruction of proximal portion of female genitalia. L.C. = Laurer's canal; Ov. - ovary; U.D. = uteroduct; V.D. = vitelline duct; Y.D. = yolk duct; Y.R. = yolk reservoir.

B. Egg from mouth of heron.

The eggs (Fig. 6b) are oval, operculated with a relatively thick brown shell; when laid their contents are still in the morula stage. They vary in length from 0.115 to 0.122 mm. by 0.07 to 0.073 mm. broad. The vitellaria consists of numerous isolated glandular follicles extending from behind the acetabulum to the posterior end in two broad lateral bands along the sides of the body except that they meet ventrally under the uterine sac and posterior of the genitalia; two transverse ducts, one on each side, pass inwards and unite with the corners of the yolk reservoir.

### DISCUSSION.

From the above description it will be noted that the morphology is very similar to that of *C. lophophallum* Baer, 1933; it, however, differs from this species in that a pharynx is absent and the intestinal caeca open to the exterior through the excretory pore. These two characters together are of sufficient importance to separate this species from all the known species of this genus, and the writer has great pleasure in naming it after the donor through whose interest and kind services the material was made available for study.

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Since writing the above, the writer has received a personal communication from Dr. J. G. Baer, wherein he informs the writer that he has re-examined his sections of *C. lophophallum* and has failed to observe a communication between the intestine and excretory vesicle. He has, however, found some red blood corpuscles in some sections of the excretory canals, and therefore suggests that some communication must exist. A re-examination of the writer's section has failed to reveal any red blood corpuscles in the excretory canals.

## Section IV.

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# Bacteriology.

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MASON, J. H. . . . . The lethal dose of the toxins of some anaerobes for sheep.

MASON, J. H., AND ROBINSON, E. M. The antigenic compounds of the toxins of *Cl. botulinum* types C and D.



## The Lethal Dose of the Toxins of Some Anaerobes for Sheep.

By J. H. MASON, F.R.C.V.S., F. R.S.E., Empire Marketing Board  
Research Fellow, Onderstepoort.

A COMMON method of determining the immunity produced in sheep by the injection of antigens prepared from the sporulating anaerobes is the intramuscular or subcutaneous injection of living culture. Provided that one has had considerable experience with the antigen and the organism in question, little objection can be raised—a rough answer, usually sufficient for practical purposes, is obtained. However, not infrequently the test culture, prepared in the same manner as on previous occasions, fails to kill the controls or test animals that should die, and thus the experiment is rendered valueless.

With the exception of *Cl. chauvoei* (causing black quarter), the anaerobes responsible for diseases in sheep are toxin producers. These are *Cl. septicus* (braxy), the lamb dysentery bacillus or *Cl. welchii*, Type B, Wilsdon (lamb dysentery, bloodpens), *Cl. oedematiens* (black disease), and *Cl. oritoxicus* or *Cl. welchii*, Type D, Wilsdon (enterotoxaemia and pulpy kidney disease).

Since the toxins of the anaerobes are readily stabilized by precipitating toxic filtrates with ammonium sulphate and drying, and, further, since the injection of toxin is a more accurate method of testing antitoxic immunity than is the injection of culture, the writer decided to compare the toxicity of the various toxins in sheep and in mice and guinea-pigs. The results are presented here, in the hope that they may serve as a guide to others who have not had a sufficient number of sheep at their disposal to establish the ratio between toxicities in mice and sheep.

All the organisms were "single-celled" prior to the commencement of the test. Morphologically, culturally, biochemically, and as regards their pathogenic effect on guinea-pigs, they behaved in the manner described for typical strains. The *Cl. septicus* and *Cl. oedematiens* cultures were originally obtained from the National Collection of Type Cultures, Lister Institute, the *Cl. oritoxicus* strain ( $R_2$ ) from Dr. Bennetts, and the lamb dysentery organism was a sub-culture of that originally isolated by Major Dalling. It should be noted that this Type B strain was of the "1930 variety" (Montgomerie and Rowlands, 1934; Dalling, 1934; Mason, 1934), and thus did not produce the *epsilon* toxic fraction of Glenn *et alia* (1933).

All cultures were grown at 37° C. in Robertson's meat broth (horse flesh) for the following periods: Type B, 20 hours; *Cl. septicus*, 36 hours; *Cl. oedematiens*, 48 hours; and *Cl. oritoxicus*, 5 days. After filtration, first through pulp and then through a

# LETHAL DOSE OF THE TOXINS OF SOME ANAEROBES FOR SHEEP.

Berkefeld candle, the filtrates were saturated with ammonium sulphate, the precipitate blotted and finally dried *in vacuo* over  $H_2SO_4$ . For use 100 mgm. were dissolved in 5.0 c.c. of saline solution.

The minimum lethal dose for mice and guinea-pigs was established in hundreds of animals. The *Cl. septique* and Type B toxins had been prepared seven years previously, their toxicities remaining constant over this period. The oedematis toxin (toxicity worked out by the intramuscular injection of mice) and that of ovitoxic were of recent preparation, their values being established over a period of months on approximately 70 mice.

*The Sheep.*—All were Merinos, mostly hammels and of different weights and ages. Nearly all had been through a louping-ill experiment; some through blue-tongue, black quarter, or various plant-feeding experiments. After the injection (always intravenous) of toxin, at least a week was allowed to elapse before the result was recorded. In some cases, sheep received three different toxins at different times. However, sheep which survived the injection of *Cl. welchii*, Type B toxin never received that of *Cl. welchii*, Type D and *vice versa*.

When experimentation indicated the approximate fatal dose, further tests were put up in which so much toxin per kilo-body-weight was given.

## RESULTS.

### *Cl. septique.*

Number of sheep used: 38.

Weights varied between 20.4 and 32.0 kilograms.

### TOXICITY.

Irrespective of Weight.		Per Kilogram Body weight.		Effect of injecting 2.6-3.3 mgm. per kilogram.
Mgm.	Result.	Mgm.	Result.	Weight (Kg.).
120	†	4.4	†	Less than 22
110	†	3.8	†	†††††
100	†/	3.5	†/	22-25 †††††
				25.3-27.5
90	†††/	3.3	††	†††††††
				28 or more
80	††††/	3.0	††††//	††††††
72, 74, 76	††††//	2.8	†//	
70	†††/	2.6	††††††††//	
66	††/	2.3	/	
60, 62	††/	2.2	†††//	
54	†/	1.7	///	
44, 46, 48, 50	†//†††/			
40	†/			

M.L.D. mice (15-18 gms.) 0.1-0.2 mgm.

M.L.D. guinea pigs (250-300 gms.) 3.0 mgm.

† death.  
/ = live.

To be certain of killing a mouse, 0.2 mgm. was necessary, although 0.1 mgm. frequently did so. The same variation occurred in sheep, some being killed with 2.2 mgm. per kilogram and some living after having received 3.0-3.5 mgm. per kilogram. It would appear that more than 3.0 mgm. per kilogram was necessary to be reasonably certain of a lethal effect. Taking the lethal doses at 0.2 mgm. and 3.5 mgm. per kilogram for the mouse and sheep respectively, the ratio of mouse to kilogram sheep-body-weight is 1 : 17.5.

The time elapsing between injection and death varied between four hours (3.5 mgm. per kilogram) and 30 hours (2.6 mgm. per kilogram). However, as a rule, sheep injected during the day with a lethal dose were dead next morning.

*Cl. welchii*, Type B.

Number of sheep used: 18.

Weights varied between 12 and 32 kilograms.

#### TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
20-24	++	1.1-1.55	+++
16-17	++++	0.75-0.9	+++//
15	/	0.5-0.65	++++//
10-12	+++//	0.35-0.45	////
7			
M.L.D. mice (15-18 gms.) 0.02 mgm.		+ death.	
M.L.D. guinea pigs (250-300 gms.) 0.14-0.2 mgm.		live.	

Taking the sheep M.L.D. to be 1.2 mgm. per kilogram, the mouse to sheep ratio is 1 : 60. The interval between injection and death varied between one hour (1.1 mgm. per kilogram) and four days (0.66 mgm. per kilogram). Four sheep died within four hours, three in from 18 to 36 hours, and three in from 3 to 4 days.

*Cl. welchii*, Type D.

Number of sheep used: 23.

Weights varied between 14 and 35 kilograms.

#### TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
100	+	6.6	+
10, 15, 20, 40	++++	2.5	+
7, 8	++	0.7, 0.75, 1.0	+++
4, 5	+/	0.25	++
2.0-2.5	++++	0.14, 0.17	++
1.8	/	0.09-0.1	++++
1.0	///	0.05, 0.065	///
0.5, 0.7	//	0.04	///
		0.025	/
M.L.D. mice (15-18 gms.) 0.008 mgm.		† = death. / = live.	



# LETHAL DOSE OF THE TOXINS OF SOME ANAEROBES FOR SHEEP.

*Cl. welchii*, Type D toxin gave a sharper end point in sheep than either *Cl. septique* or *Cl. welchii*, Type B, because, with the exception of one sheep, all which received 0.1 mgm. (or more) per kilogram died, whereas those injected with 0.065 mgm. (or less) per kilogram lived. Taking 0.1 mgm. per kilogram as the lethal dose, the mouse: sheep ratio is 1:12.5.

The toxin killed quickly, 0.25 mgm. (or more) per kilogram killing in 1.4 hours. Doses around the M.L.D. killed in less than 24 hours, i.e. overnight the animals were dead, only one sheep (0.14 mgm. per kilogram) surviving this period, to die, however, within 48 hours. However, even after 24 hours this animal was moribund.

*Cl. oedematiens*.

Number of sheep used: 6.

Weights varied between 19 and 24.5 kilograms.

## TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
200	†	8.5	†
150	†	8.0	†
150	†	7.5	†
150	/	4.0	/
50	/	2.0	/
10	/	1.0	/

M.L.D. mice (15-18 gms.) intramuscular injection—0.06-0.08 mgm.

† = death. / = live.

The small number of sheep used does not allow the drawing of conclusions, but assuming 7.0 mgm. per kilogram to be a lethal dose for sheep, the mouse to sheep kilogram ratio is in the region of 1 : 100.

The three animals which succumbed, died within 24 hours.

## SUMMARY.

The lethal dose of the toxins of four anaerobes has been ascertained in mice, sheep, and in two instances in guinea-pigs also. The mouse : kilogram-body-weight-sheep ratio is as follows:—

*Cl. septique* 1:17.5, *Cl. welchii*, Type B 1:60, *Cl. welchii*, Type D 1:12.5 and *Cl. oedematiens* 1:100. The last ratio was arrived at by the use of only six sheep and is thus approximate.

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## **The Antigenic Components of the Toxins of *Cl. botulinum* Types C and D.**

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### **INTRODUCTION.**

INTOXICATIONS caused by the toxins of the C and D types of *Cl. botulinum* have been reported from different parts of the world (America, Australia, Tasmania, and South Africa), the diseases produced usually being associated with the consumption of carrion or decomposed material. Although the fact that the C and D types do cause intoxications in the domestic animals is well established, the relationship of the toxins of the C type to another and their relationship to the D type is not in the same position. Bengtson (1922, 1923) reported that toxic filtrates of the C type organism isolated from fly larvae were not neutralized by A or B type antitoxin and that C antitoxin did not neutralize A or B toxins. Seddon (1922), discussing his *parabotulinus* bacillus, noted that there was no cross protection between it and the A or B types. Pfenniger (1924) stated that 0.2 c.c. to 0.5 c.c. of C antitoxin neutralized two fatal doses (for guinea-pig) of C toxin, but that this amount of toxin was not neutralized by A, B, or Seddon antitoxins. Further, Seddon toxin was neutralized by his C antitoxin, but not by A or B.

Doyle (1923) noted that the toxin associated with limberneck in chickens, investigated by him, was not neutralized by A or B type antitoxins. Graham and Boughton (1924) were able, with antitoxins produced with type C toxin, to neutralize Seddon toxin, and, further, antitoxin made with the toxin produced by limberneck (type C) germs neutralized homologous, heterologous, and fly larvae toxins. As already reported (Robinson, 1930), one of us (E.M.R.) showed that the South African C type toxin (equine origin), a C type toxin (Graham, U.S.A.), and the Seddon toxin cross-neutralized; however, D antitoxin did not neutralize any of these toxins. Further, in a personal communication, Graham stated that his C antitoxin neutralized *Cl. parabotulinum* toxin (i.e. C type toxin of South African equine origin). Meyer and Gunnison (1929), examining cultures, received from this institute, stated that the D type toxin was not neutralized by A, B, or C antitoxins. Gunnison and Meyer (1932), working with a disease in ducks associated with *Cl. botulinum* type C, showed that antitoxins prepared from the duck strain and

from strain 526 Ca neutralized the duck, 526 Ca, Seddon, and 550 Cb toxins, whereas Seddon (Cb) antitoxin neutralized only the homologous and the 550 Cb toxins. Bennetts (1933) showed that the toxin prepared from an impure culture of a type C strain was neutralized by the Australian parobotulinum antitoxin and by C antitoxin. Theiler and Robinson (1928) stated that the D type antitoxin (5.0 c.c.) did not neutralize two lethal doses of the South African C, the Seddon, American C, or the A type toxins.

From the foregoing it is clear that unanimity of opinion does not exist as regards the relationship of the C toxins to one another. The possibility exists that a cause of this may be that the antitoxins used were of very low titre. This opinion is supported by the fact that large amounts of antitoxin (up to 5.0 c.c.) were required to neutralize only a few fatal doses of toxin. However, the literature would indicate that the D type (South African bovine, associated with lamsiekte) toxin is monospecific.

In view of the lack of uniformity of results and as work on another group of anaerobes, the *Cl. welchii*-*Cl. ovitoxicus* group, showed that the toxins produced by individual members may share the same toxic fractions [Wilsdon (1931), Glenny *et alia* (1933), Montgomerie and Rowlands (1934), Dalling (1934), Gill (1933)], work was commenced on six C type, one D type, and one A and one B type toxins. In the authors' opinion, the results obtained show that in the C and D groups there is, as in the *Cl. welchii* group, a sharing of toxic antigenic fractions.

## METHODS.

*Origin of Cultures.*—The cultures 26C, Seddon, and D were all impure, the contaminants being proteolytic non-pathogenic anaerobes. That the only toxin-producing germs contained therein were of the botulinus type was shown by the fact that one certain lethal dose of toxin (for mouse) was not neutralized by 0.1 c.c. of the antitoxins of *Cl. septique*, *Cl. welchii*, *Cl. oedematiens*, *Cl. histolyticum*, *Cl. tetani*, *Cl. ovitoxicus*, and the "lamb dysentery bacillus", or by a mixture of 0.025 c.c. of each of them. A determined attempt to purify the D strain failed. One of us (E.M.R.), some years ago, was able to reduce the number of concomitant micro-organisms to two, and after further work eventually sealed off the culture as pure. However, on subculturing this sealed-off culture two years later, the presence of at least two non-pathogenic anaerobes was noted.

The other C and D strains, all received from Dr. K. F. Meyer, California, appeared, on microscopical and cultural examination, to be pure cultures.

The A and B strains gave no indication of impurity.

26 C.—A South African equine strain isolated at these laboratories from a decomposed rat.

Seddon.—A strain received from Dr. Seddon, Australia.

*D.*—A “bovis” strain, isolated from a bovine dead from lambsiekte, Armoedsvlakte.

*A and B.*—Received from the Lister Institute, London.

Strain 165, Type D: from K. F. Meyer. Labelled “South African Bovine”.

Strain 178, Type C: from K. F. Meyer. Labelled “South African 550”.

Strain 191, Type C: from K. F. Meyer. Labelled “Bengtson 526”.

Strain 192, Type C: from K. F. Meyer. Labelled “Seddon strain from Australia”.

Strain 205, Type C: from K. F. Meyer. Labelled “Bengtson 1187”.

Strain 165 D proved non-toxic, as did a further culture of the same organism received from Dr. Meyer.

*Preparation of Toxins.*—Robertson's meat broth (horse muscle extract, 1 per cent. peptone, 0.5 per cent. NaCl; the flask or tube containing boiled meat to about one-third the volume), plus 5 per cent. sterile horse serum was used in all cases to produce the toxin. The medium (pH 7.6) was boiled for two hours, cooled quickly, the serum added, inoculated with a 48-hours' culture of the germ, and incubated for seven days at 37° C. The culture was then rendered sterile by passing it through, first, a paper pulp and then a Berkefeld filter candle.

To render the toxin stable the toxic filtrate was saturated with ammonium sulphate (about 55-60 grams per 100 c.c.) and the resultant precipitate pressed, dried *in vacuo* and powdered. Such powder, dissolved in saline, maintained its original (dry) value throughout the experiment.

*Production of Antitoxin.*—Goats proved suitable for this purpose, being cheap in price and large enough to give a sufficiency of serum for the number of tests carried out. Formol-toxoid was employed in the initial immunization, being prepared by adding 0.3 per cent. commercial formalin (40 per cent. formaldehyde) to the pulped (not candled) toxin at pH 7.4, and incubating this at 37° C. until 0.1 c.c. did not kill a mouse on subcutaneous inoculation. The time necessary for this to occur varied between 1 and 5 weeks. It was found that it was essential to reach this degree of non-toxicity before commencing immunization; on two occasions, the use of a C (South African) equine toxin, the toxicity of which was such that 0.1 c.c. killed a mouse in three days (0.05 c.c. being non-lethal), killed two goats when a dose of 2.0 c.c. was reached. When a dose of toxoid between 20 c.c. and 30 c.c. had been reached, one could with impunity double the dose weekly or change over to unmodified toxin. The final 6-8 inoculations were of toxin, each dose being between 100 c.c. and 200 c.c. in amount. Unless the condition of the goat did not warrant it, the injections were made twice weekly, the previous dose being increased 50 per cent. and later 75 per cent. or 100 per cent.

The course of immunization lasted between two and three months. Trial weekly titrations of bleedings were made, commencing at about the second month; when no rise in antitoxin over the previous one was detected, the animal was exsanguinated, the serum collected from the clot and preserved with 0.5 per cent. phenol. One antitoxin, D(r), was prepared, some years ago, by one of us (E.M.R.) in a horse by the use of the D strain toxin.

*Titrations of Toxin and Antitoxin.*—Mice of about 15-18 grams were employed as test animals, the route of injection being the subcutaneous. Whilst it is realized that the guinea-pig has been the test animal of choice for the titration of botulinus toxin and antitoxin, the mouse proved to be quite as accurate an indicator, and further was cheaper, more easily housed in large numbers, and obtainable in uniform body weight.

Dry toxin was dissolved in saline in the proportion of 100 mgm. to 5.0 c.c. and from this solution dilutions again made in saline. The approximate minimal fatal dose was then worked out, and whilst no attempt was made to ascertain this very accurately, sufficient mice were injected around the probable L.D.<sub>50</sub> (Trevan) point to be reasonably certain that a certain fatal dose could be predicted. The end point was sufficiently sharp, with all toxins at about the M.L.D. level to allow a 30 per cent. drop in toxin amount to result in a "live" or "paralysed" instead of a "death".

For antitoxin titrations, a test dose of toxin was chosen after a few preliminary trials. Beyond ensuring that this dose contained at least 10 sure fatal doses no attempt was made to have the same number of lethal doses in the test dose of each toxin. This dose, mixed with varying amounts of antitoxin, was left for one hour at room temperature (20°-26° C.) and injected into mice. Where preliminary titrations showed that a serum was poor or deficient in antitoxin, one certain fatal dose of toxin was mixed with from 0.1 c.c. to 0.5 c.c. of antitoxin and injected.

In all cases, observation was carried on for seven days, results being recorded as "lived", "paralysed", or "dead".

## EXPERIMENTAL.

In the first series of titrations, the neutralizing power of the antitoxins of 26 C, Seddon, and D [including D(r)] was tested against toxins made from these strains. An example of such a test is given in Table 1.

TABLE 1.

<i>Toxin:</i> 26C.	<i>MLD:</i> 0.0003 c.c.	<i>T.D.:</i> 0.01 c.c.
<i>Antitoxin:</i> Seddon.		
<i>Dose (c.c.)</i>		<i>D. P. L.</i>
0.0007.....		4 0 0
0.0008.....		3 1 0
0.001.....		0 1 4
0.0012.....		0 0 3

(M.L.D.=minimum lethal dose. T.D.=test dose. D=died. L=lived. P=paralysed, i.e. showed paralysis during the seven days' observation but survived seven days. In this titration 0.001 c.c. of antitoxin is taken as a neutralizing dose.)

Similar titrations were carried out with all the toxins and antitoxins, at least three and usually six to eight mice being injected at those levels just around the neutral point. Table 2 summarises the titrations of the toxins of 26 C, Seddon, and D against the antitoxins of these strains. The central unenclosed figure is the neutralizing dose of antitoxin.

TABLE 2.

Toxin.	MLD.	T.D.	Antitoxins.			
			26 C.	S.	D.	D(r).
26 C	0.0004	0.01	(3-3½) 0.003- 0.0035	(1)  0.001	(50)  0.05	(200-240) 0.1- 0.12
			【1】	【1】	【1】	【1】
			(4) 0.005- 0.006	(1)  0.0015	(66)  0.1	(5½)  0.08
S	0.0001	0.01	【1½-2】	【1½】	【2】	【4】
			(1½) 0.02- 0.025	(1) 0.03- 0.035	(1/600)  0.02	(1/440)  0.025
			【600】	【1200】	【2/5】	【1/8】

T.D. of D toxin v 26 C and S antitoxins = 0.00001 and 0.000025 c.c. respectively.

T.D. of 26 C toxin v D(r) antitoxin = 0.005 c.c.

T.D. of S toxin v D(r) antitoxin = 0.001 c.c.

All unenclosed figures = c.cs.

Figures in 【 】 = ratio of one toxin to another.

Figures in ( ) = ratio of one antitoxin to another.

Central unenclosed figure = neutralizing dose of antitoxin.

The conclusions to be drawn from Table 2 are (1) the toxins 26 C and Seddon are antigenically the same and (2) that D toxin contains some C antigen and the C and Seddon toxins some D antigen, but that the amount of heterologous antigen contained in each toxin varies greatly, as does the amount of heterologous antitoxin contained in each serum.

In table 3 the results of titrating fresh brews (precipitated with ammonium sulphate and dried as before) of 26 C and Seddon toxins against antitoxins of 192 C, 178 C, 191 C, and 205 C are summarized. The data given therein confirms the results shown in Table 2, viz., that the toxins, 26 C, and Seddon, are antigenically the same.

# ANTIGENIC COMPONENTS OF THE TOXINS OF "CL. BOTULINUM

TABLE 3.

Toxins.	MLD.	T.D.	Antitoxins.			
			192	178	191	205
26C	0.001	0.01	(12) 0.006 【1】	(7) 0.0035 【1】	(12) 0.006 【1】	(1) 0.0005 【1】
S	0.00025	0.01	(8-10) 0.02- 0.025 【4】	(4-6) 0.01- 0.015 【4】	(8-10) 0.02- 0.025 【4】	(1) 0.0025 【5】

Central unenclosed figure = neutralizing dose of antitoxin (c.c.).

All unenclosed figures = c.c.

Figures in 【 】 = ratio of one toxin to another.

Figures in ( ) = ratio of one antitoxin to another.

In Table 4 is summarized the titration of the toxins of 192 C, 178 C, 191 C, and 205 C against the six C antitoxins.

TABLE 4.

Toxins.	MLD.	T.D.	Antitoxins.					
			26 C.	Seddon.	192 C.	178 C.	191 C.	205 C.
192 C.	0.0005	T.D. =	0.1 (3-4) 0.01 【1/3】	0.1 (1) 0.0025- 0.003 【1/4-1/3】	0.025 (27) 0.017 【1/3】	0.025 (13-16) 0.01- 0.012 【1/3】	0.1 (10-12) 0.02- 0.03 【1/4】	0.1 (1½-1½) 0.004 【1/3】
178 C.	0.0001	T.D. =	0.1 (3½) 0.035 【1】	0.1 (1) 0.01 【1】	0.01 (25) 0.025 【1】	0.01 (10-12) 0.01- 0.012 【1】	0.01 (17) 0.017 【1/5】	0.1 (1½) 0.015 【1½】
191 C.	0.0004	T.D. =	0.01 (4) 0.035- 0.045 【1-1½】	0.1 (1) 0.01 【1】	0.002 (5) 0.01 【2】	0.01 (10) 0.1 【5/6】	0.01 (½) 0.004- 0.005 【1/30】	0.01 (½) 0.0025 【1/5-1/4】
205 C.	0.00015	T.D. =	0.1 (3) 0.03- 0.035 【1】	0.1 (1) 0.01- 0.012 【1】	0.01 (20-25) 0.025 【1】	0.01 (10-12) 0.012 【1】	0.01 (10-12) 0.012 【1】	0.01 (1-1½) 0.012 【1】

All unenclosed figures = c.c.

Top unenclosed figures opposite T.D. = test doses used.

Figures in 【 】 = ratio of one toxin to another.

Figures in ( ) = ratio of one antitoxin to another.

Central unenclosed figures = neutralizing dose of antitoxin.

From Tables 3 and 4 the following information may be gathered: the toxins 26 C, Seddon, 192 C, and 178 C are antigenically indistinguishable. As, however, 192 C and 178 C are merely purified cultures of 26 C and Seddon respectively, one can say that, by the methods employed, no difference in the antigenic constitution of their toxins can be elucidated. Further, reference to Table 2 indicates that the amount of the D toxin fraction of their make-up does not differ much, if at all. These two toxins and the two Bengtson strains, 191 C, and 205 C, share two antigens, one of which may be termed the "main" and the other the "subsidiary" fraction. In the elimination of the 191 C toxin titration from Table 4, the subsidiary fraction would remain unrevealed, and the other three toxins would be considered identical. It is obvious from the table that the expected ratios are not obtained with the use of 191 C toxin; three and possibly four figures are "out", two of them beyond the limits of experimental error and of the method of testing. If the toxin molecule be considered as containing three fractions C<sub>1</sub>, C<sub>2</sub>, and D, then the apparent discrepancy obtained with the use of 191 C toxin may be explained. The particular brew employed contained an excess of the C<sub>2</sub> fraction and, as follows, the antitoxin produced by it contained an excess of C<sub>2</sub> antitoxin.

As there was the possibility that 191 C toxin readily dissociated, *in vivo*, from a toxin-antitoxin mixture, a number of tests were put up to check the point. On three different occasions, mixtures were made, and their effect on mice ascertained after they had stood for one hour at room temperature and at 5° C. for 24 hours. The results did not differ materially from those recorded in Table 4.

In table 5 are recorded the results of the titration of four C type toxins against the D and D(r) antitoxins.

TABLE 5.

Antitoxins.	Toxins.				
	T.D.	192 C.	178 C.	191 C.	205 C.
D	T.D. --	0.01	0.001	0.0025	0.005
		<b>1</b>	<b>21</b>	<b>1</b>	<b>1</b>
		0.05 (1)	0.02 (1)	0.12 (1)	0.1 (1)
D(r)	T.D. --	0.005	0.001	0.0005	0.001
		<b>1</b>	<b>1</b>	<b>=</b>	<b>1</b>
		0.1 (1)	0.15 (1/7)	> 0.3 (=)	0.1 (1/5)

T.D. = Test dose of toxin used. (c.c.)

Central unenclosed figure = neutralising dose of antitoxin (c.c.).

Figures in **[ ]** = ratio of one toxin to another.

Figures in ( ) = ratio of one antitoxin to another.

From the data given in Table 5 it is apparent that the amount of C antitoxin contained in the two D sera is not the same, in each instance D(r) being much the poorer; it will be seen that 0.3 c.c.



of D(r) antitoxin was unable to neutralize one M.L.D. of 191 C toxin. The possibility exists that this discrepant result may be explained by the amounts of the C<sub>1</sub> and C<sub>2</sub> fractions contained in the toxins and antitoxins. As Table 4 shows, 191 C toxin contains an excess of the C<sub>2</sub> fraction; the lack of the anti-C<sub>2</sub> fraction in D(r) antitoxin would explain its inability to neutralize.

Table 2 showed that cross-neutralization existed between the toxins and antitoxins of 26 C, Seddon, and D. Using a fresh brew of D toxin, a series of tests were put up to ascertain the neutralizing power of all the C antitoxins against it. Table 6 summarizes the results.

TABLE 6.

(M.L.D. of new D toxin = 0.000002 c.c.)

*Antitoxins (c.c.).*

	26 C.	S.	205 C.	192 C.	178 C.	191 C.
Toxin D (c.c.)	0.1 neutralized 0.000007- 0.00001	0.1 neutralized 0.00001- 0.000015	0.1 neutralized 0.00001- 0.000015	0.5	0.5	0.5
				Did not neutralize one M.L.D.		

The antitoxins 26 C, Seddon, and 205 C in doses of 0.1 c.c. were able to neutralize a few M.L.D. of D toxin, but the three other C sera, even in doses of 0.5 c.c. were incapable of neutralizing one fatal dose. The antitoxic fraction of 191 C serum was salted out by half saturation with ammonium sulphate; the precipitate was dialysed against distilled water for three days and the inner fluid fanned down to one-quarter of the original volume. Although the anti-C (191) value of this concentrate was three times that of the original, 0.5 c.c. was incapable of neutralizing one M.L.D. of D toxin. The possibility existed that the goats used to produce 192, 178, and 191 antitoxins did not receive enough of the D fraction (presumed to be present in small quantity in the C toxic filtrates) to give rise to the production of D antitoxin in demonstrable amount. Further, the C fraction of the toxin so preponderated over the D that unless the animal body had exhausted its power of producing C antitoxin, there would be little chance of D antitoxin being produced in appreciable quantity. Immunization was therefore undertaken in three new goats, and carried on for three months, finishing up the process by injecting 100-200 c.c. toxin ten times within three weeks. The 192 goat was then bled out, but the other two were rested for a fortnight and then "shocked" by injections of 100-200 c.c. of toxin given every second day for ten days. By this method antisera were obtained containing demonstrable amounts of D antitoxin in the case of 191 and 178 antitoxins. Table 7 gives the results.

TABLE 7.

(M.L.D. of D toxin = 0.000002 c.c.)

*Antitoxins.*

	191 C.	178 C.	192 C.
Toxin D	0.5 c.c. neutralized 0.000004 c.c.— 0.000005 c.c.	0.1 c.c. neutralized 0.000015 c.c.	0.5 c.c. did not neutralize one M.L.D.

As a control to the large dose of serum (0.5 c.c.) used, as shown in Table 7, experiments were carried out using 0.5 c.c. of normal goat serum and 0.5 c.c. of antitoxin prepared in goats against *Cl. welchii*, Type B, *Cl. chauvoei*, *Cl. ovitoxicus*, and *Cl. septicæ*. In no instance did this amount neutralize one sure lethal dose of D toxin. The results given in Table 7 show that the toxins of 191 C and 178 C contain a small amount of D toxin; the failure of 192 antitoxin may mean either that its toxin contains none of the D type toxin or that immunization was not carried far enough to allow of the production of demonstrable amounts of antitoxin.

The A and B type toxins appeared to be monospecific. A test dose (about 20 M.L.D.) of A toxin was neutralized by 0.015 c.c. of its own antitoxin, whereas 0.2 c.c. of B, C (26 C, S, 191 C, 192 C, 178 C, and 205 C), and D antitoxins failed to neutralize one fatal dose. Further, from 0.1 c.c. to 0.2 c.c. of A antitoxin did not neutralize one lethal dose of the toxins of the B, D, and the various C toxins. In the same way, B toxin was neutralized by its own antitoxin, one test dose, about 100 lethal doses, being rendered non-toxic by 0.01 c.c. of antitoxin; however, its toxin (one M.L.D.) was unaffected by the other antitoxins and its antitoxin had no neutralizing power on the other toxins.

**DISCUSSION.**

The results just recorded show that the toxins of the C and D botulinus group contain more than one toxic component. That there are three antigens concerned is fairly obvious—the 2 C fractions, C<sub>1</sub> and C<sub>2</sub>, and the D. However, no proof has been presented that these are the only antigens present, this investigation merely showing that, by ordinary methods and reasonably careful technique, some of the fractions making up the toxic molecule may be demonstrated. It is realized that a great deal more work would have to be done in order to arrive at anything approaching finality on the question. To explain an apparent discrepancy, the hypothesis has been formulated that the C toxin is composed of a C<sub>1</sub> and a C<sub>2</sub> fraction. Assuming this to be correct, there is the definite possibility, arguing from the analogy of the *Cl. welchii-ovitoxicus* group, that the proportion of the C<sub>1</sub> and C<sub>2</sub> fractions produced in culture would vary, depending upon the age of the culture. As an example it may be stated that *Cl. welchii*,

Type B, produces, in the early stages (12-24 hours) of growth only the *Cl. paludis* type of toxin, whereas if growth is allowed to continue, this diminishes greatly, to be replaced in large measure by the *Cl. oritoxicus* type of toxin.

Further, as has been shown in the *Cl. welchii* group, the animal to be immunized also enters into the question; depending upon its "natural" immunity and no doubt also on factors unknown, the antitoxin produced will differ qualitatively as well as quantitatively. Thus, it is obvious, arguing from such a standpoint, that this investigation serves merely as a pointer to further work.

A second and obvious explanation for the sharing of antigens is that the cultures were mixtures of more than one C and the D types. This is a possible but extremely improbable explanation. Against it are the following facts: (1) the cultures originated in Australia, South Africa, and America; it is unreasonable to suggest that in each case the C (two varieties) and the D types were picked up; (2) there is some regularity in the anti-C content of D sera and the anti-D content of C sera (Tables 2, 5, 6, and 7). It will be seen that 0.02 c.c. to 0.1 c.c. of D antitoxin neutralizes 6 to 20 fatal doses of C toxin, whereas from 0.1 c.c. to 0.5 c.c. of the C antitoxins was necessary to neutralize two to seven lethal doses of D toxin. In toxic filtrates made from a mixture, one would expect greater variation of the C and D fractions, as judged by the anti-C and anti-D content of the sera. As, in the production of the antitoxins, several (2 to 5) different brews of toxin were used to produce each antitoxin and as the anti-C content of the D sera and the anti-D content of the C sera was always very low, it would appear unlikely that a mixed culture is the explanation of the sharing of antigens. Again with regard to the C toxins—if the irregularity obtained with 191 C toxin and antitoxin is to be explained by assuming contamination, then all the C cultures contained this contaminant. As before stated, such an occurrence is extremely unlikely.

#### CONCLUSIONS.

Toxic broth filtrates of one A, one B, six C, and one D types *Cl. botulinum*, and the antitoxins produced by injecting these filtrates into goats have been tested, from the standpoint of working out the antigenic "make up" of the toxins. The results obtained indicate:—

- (1) The A and the B types are monospecific.
- (2) The C types contain three components (a) C<sub>1</sub>, (b) C<sub>2</sub>, and D. The D fraction is contained in only very slight amount.
- (3) The D type contains chiefly the D, but also a small quantity of the C fraction.

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## Section V.

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# Plant Studies AND Poisonous Plants.

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- RIMINGTON, C., AND     Note upon the isolation of the toxic prin-  
SIEYN, D. G.             ciple from a species of *Dimorphotheca*,  
                               probably *Dimorphotheca fruticosa*.
- RIMINGTON, C. ...     Chemical investigations of the " Gifblaar "  
                               *Dichapetalum cymosum* (Hook) Engl. I.
- LÉEMANN, A. C. ...     Hydrocyanic Acid in Grasses.



## **Note upon the Isolation of the Toxic Principle from a Species of *Dimorphotheca*, probably *Dimorphotheca fruticosa*.**

By

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*Registered Number:* Onderstepoort Spec. No. 9066: 25.7.34.

*Origin:* Heroncliff, Port Elizabeth.

A PLANT reported to have caused poisoning of stock in the Port Elizabeth district, was recently submitted to this Laboratory for examination. The material was in the flowering state. Specimens were sent to the Division of Plant Industry for botanical identification, but, unfortunately, no seeds were available. The Principal Botanist identified the plant as a species of *Dimorphotheca*, probably *Dimorphotheca fruticosa*.

This species has not previously been chemically examined, although the cyanogenetic glucoside linamarin has been isolated from *Dimorphotheca spectabilis* and *Dimorphotheca zeyheri* (Rimington, 1932), from *Dimorphotheca cuneata* (Marais and Rimington, 1934), and from *Dimorphotheca ecklonis* (Rosenthaler, 1922).

Tests showed the plant to be cyanogenetic and therefore hydrogen cyanide determinations were carried out and linamarin looked for, with the result that the toxic glucoside was isolated in a yield corresponding to 84 per cent. of that theoretically possible.

### **DETERMINATION OF HYDROGEN CYANIDE.**

The technique employed was that previously described (Rimington, 1932), six hours' maceration being found sufficient for complete liberation of hydrogen cyanide in the case of ground, dried material



## ISOLATION OF TOXIC PRINCIPLE FROM A SPECIES OF DIMORPHOTHECA.

and about 18 hours for the fresh cut-up plant. The results obtained were as follows:—

Fresh plant.	Moisture content.	Mgm. HCN per 100 gm.	Gm. HCN per 100 gm. dry wt.
Leaves (wilted).....	83.27	243.0	1.45
Stems.....	73.24	82.8	0.31

Dried, powdered material contained 278 mgm. HCN. per 100 gm. There was thus considerable loss on drying.

## ISOLATION OF THE CYANOGENETIC GLUCOSIDE.

The method employed was direct extraction in a Soxhlet apparatus with ethyl acetate. The extract was evaporated to dryness, taken up in water, filtered, again evaporated in presence of calcium carbonate and a little decolorising charcoal and the dry residue boiled up repeatedly with dry ethyl acetate. The glucoside separated in pure form on cooling this extract. In all, there was obtained 4.2071 gm. from 200 gm. of starting material, a yield equivalent to 84.2 per cent. of that theoretically possible.

It had all the properties of linamarin and a specimen did not depress the melting point of authentic linamarin isolated from *Dimorphotheca spectabilis*.

The following constants were recorded:—

$$\begin{aligned} \text{M.P. } 141^{\circ}. \quad & \cdot \left[ \alpha \right]_{\text{D}}^{21} = - \frac{0.67 \times 15 \times 100}{2 \times 19.91} \\ & = - 25.23^{\circ} \end{aligned}$$

## SUMMARY.

The cyanogenetic glucoside linamarin has been isolated from a species of *Dimorphotheca*, probably *Dimorphotheca fruticosa*. The leaves of the plant contain approximately 1.5 gm. of HCN. per 100 gms.

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## **Chemical Investigations of the "Gifblaar" *Dichapetalum Cymosum* (Hook) Engl. I.**

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Fellow under the Empire Marketing Board.

### THE PLANT AS A STOCK POISON.

THE plant *Dichapetalum cymosum* (*Chailletia cymosa*) or "Gifblaar" is one of the most well-known poisonous plants of the Transvaal. Belonging to the family Dichapetalaceae, a small tropical family of trees or woody shrubs, it is the sole representative which has developed a subterranean habit. It is an underground shrub possessing branches, many of which attain great length and from which small shoots ascend to the surface, there giving rise to the familiar tufts of green lanceolate-ovate leaves and inconspicuous flowers.

A good description of the plant is to be found in the publications of Phillips (1927) and of Mogg (1930). Leeman (personal communication) has recently distinguished several varieties. The Gifblaar has a very limited distribution in South Africa, being practically confined to the Transvaal and not occurring south of the delimitation Mafeking, Lichtenburg, Pretoria, Middelburg, nor east of Middelburg-Pietersburg, being thus confined to the lower altitudes. The problems of its distribution have been discussed by Burt-Davy (1922).

As a stock poison, the Gifblaar appears to have been known to the early settlers, and a fairly extensive literature exists, referring to its toxic properties. A certain seasonal variation in the incidence of poisoning, prompted the studies, extending over two years, undertaken by Sir Arnold Theiler and his collaborators at this Laboratory, a report of which is to be found in the article by Steyn (1928). It was shown that periods of maximum toxicity fall approximately in the seasons September-October, and March-April, respectively, but depending to some extent upon climatic conditions, times during which the plant is producing new young leaves. The bearing of soil temperatures upon this cycle has been investigated by Mogg (1930). From a stock-owner's point of view, this variation in toxicity is of importance since it is just at these two seasons that fresh, green grazing is apt to be scarce.

It has also been observed that the onset of toxic symptoms appears to be accelerated when the animal partakes of water immediately after feeding on Gifblaar. The ready solubility of the toxic principle in water has been adduced in explanation of this phenomenon.

## PREVIOUS CHEMICAL AND TOXICOLOGICAL INVESTIGATIONS.

Comparatively little work has been published upon the chemistry of the "Gifblaar", and nothing is known concerning the nature of the toxic principle. At one time, the plant was thought to contain a cyanogenetic glucoside, following upon the report by the Imperial Institute that prussic acid had been detected in the leaves, but this was not substantiated by further investigation.

Green, as stated by Steyn (1928), found the toxic principle to be thermostable, water-soluble and insoluble in chloroform or ether; furthermore, it was not precipitated by basic lead acetate nor by the usual alkaloidal reagents. Green finally obtained a pale yellow syrup which was still contaminated by sugars but toxic to rabbits in doses of 0.1 gm. per kilo when given subcutaneously.

Power and Tutin (1906) have recorded a chemical study of the fruits of the closely allied plant *Chailletia toxicaria* from Sierra Leone. They came to the conclusion that the fruit contained at least two active principles, the one causing depression or narcosis, the other, excitation leading to epileptiform convulsions. Some evidence was adduced suggesting a cumulative effect by the latter. The main part of the toxic material was soluble in water, only.

More recently, Steyn (1934) has demonstrated that an acid environment (dilute acetic acid at room temperature) has no effect upon extracts containing the active principle of Gifblaar.

## MODE OF ACTION OF THE POISON.

With regard to the mode of action of the toxic constituent, little is known with certainty. A characteristic post-mortem appearance in animals poisoned by the plant is the flabby dilated state of the ventricular walls of the heart. Power and Tutin, in *Chailletia toxicaria* poisoning, report post-mortem cerebral congestion and thrombosis of the superior longitudinal sinus.

An interesting case of human poisoning (not fatal) by *Chailletia toxicaria* is reported by Renner (1904). A native of Sierra Leone had eaten some fish dusted with the powdered fruits of the plant as a rat poison and within half an hour became ill. The symptoms were vomiting, diarrhoea, muscular twitching, weakness and loss of power to use the limbs. The tendon reflexes became abolished and some hyperaesthesia developed. Vision was slightly impaired. The man made a slow recovery during the course of two weeks but, when discharged, he still had difficulty in walking.

Gifblaar is a very serious menace to stock in those parts of the Transvaal in which it grows. Its eradication presents great difficulty on account of the underground stems, many of which may exceed 40 feet in length and be found at depths of 6 feet or more. No reliable remedy for poisoning by the plant is known.

## SCOPE OF THE PRESENT EXPERIMENTS.

The object of this communication is to report certain results obtained in a systematic study of the plant and to describe attempts which have been made to isolate the toxic principle by new methods

which have not previously been employed in this case. A good deal of indirect information has been obtained concerning the nature of the toxic principle.

The plant material used in these experiments was all collected in the Pretoria District at various times. The M.L.D. for rabbits was usually  $\pm 2.5$  gm. dry material per kilo body weight.

(a) *Extraction Experiments.*

A variety of organic solvents was employed in the hope that one could be found capable of removing the toxic principle. All extractions were made in the Soxhlet apparatus and both extract and plant residue subsequently tested for toxicity. No anhydrous solvent was found capable of removing the toxin. The following were tried with negative results: chloroform, ether, petroleum ether, carbon tetra-chloride, nitrobenzene, aniline, formamide, anhydrous acetone, absolute alcohol. The toxin was dissolved out readily by cold water.

(b) *Precipitation Experiments.*

None of the alkaloidal reagents precipitated the toxic material, neither was it removed by basic lead acetate alone or followed by ammonia, by phosphotungstic acid, mercuric chloride or acetate, flavianic acid or by any other substance tested. This would point to a neutral or amphoteric molecule possessing neither acidic nor basic characters.

(c) *Systematic Examination of an Aqueous Extract.*

An attempt was made to remove as much inert material as possible from an aqueous extract of the plant, hoping to obtain, finally, a toxic material of sufficient purity to carry out further specialised tests. The plant constituents removed at each stage were subjected to a preliminary examination and yielded certain interesting results. There were thus isolated: a tannin, a glycosidal pigment (methylpentoside), two bases, one of which proved to be the alkaloid trigonelline, a histidine-like material, and a complex polysaccharide of the uronic acid type. The detailed procedure is described below. 500 gm. of dried, ground Gifblaar leaves (M.L.D.  $\pm 2.5$  gm. per kilo) was thoroughly extracted in a percolator by water at 60-65°. To the combined extracts measuring seven litres in volume, sufficient basic lead acetate was added to produce complete precipitation. The orange-yellow precipitate was removed, washed with water and then ground with dilute acetic acid, thereby affording an orange coloured solution of an acetic acid soluble portion and a greyish insoluble residue. The soluble fraction was reprecipitated by addition of ammonia and both precipitates were decomposed by hydrogen sulphide.

TANNIN FRACTION.

The neutral lead precipitate yielded a tannin which was thrown out of solution by addition of sodium chloride. When collected on the centrifuge, washed and dried, it formed a dark brown amorphous powder.

Microanalysis: Ash 0.35 per cent.

C	H
49.60	5.42

In order to gain further insight into the nature of this substance, a portion was refluxed for one hour with 25 c.c. of a 2 per cent. solution of hydrochloric acid. The solution acquired an intense deep crimson colour and a considerable quantity of a red amorphous substance, "tannin red" or phlobaphene, separated. After cooling, the filtered hydrolysate was extracted with ether, the solvent dehydrated and allowed to evaporate. There remained a small quantity of a crystalline substance which was identified as catechol.

Crystalline material from hydrolysed tannin.

With  $\text{FeCl}_3$ , green colour turning to mauve on adding sodium acetate.

Ammoniacal  $\text{AgNO}_3$  reduced in the cold.

M.P. after recrystallisation from benzene  $103^\circ$ .

The acid liquid from which the catechol had been removed was neutralised by sodium hydroxide, extraneous material removed by lead acetate and lead by hydrogen sulphide. After concentration it was tested and found to reduce Fehlings solution on boiling.

The tannin belongs therefore to the class of catechol tannins.

#### GLYCOSIDAL PIGMENT FRACTION.

The solution remaining after removing lead from the basic lead precipitate had a deep orange-yellow colour. It was reprecipitated by basic lead acetate and the precipitate decomposed by grinding with dilute sulphuric acid. A slight excess of acid was removed from the filtrate by barium carbonate and the filtered, deep yellow solution concentrated on the water bath to the consistency of a syrup. Alcohol was then added to produce complete precipitation and the material centrifuged off and washed with ether.

It formed a bright yellow amorphous powder, moderately soluble in water and corresponding in quantity to about 1.7 per cent. of the weight of dry plant taken. It was non-toxic. With ferric chloride solution it gave a greenish coloration. On acidification of a solution the yellow colour became very much less intense.

It did not reduce Fehlings' solution.

That the substance was a glycosidal pigment was demonstrated by acid hydrolysis.

0.05 gm. was refluxed for one hour with 25 c.c. of 2 per cent.  $\text{HCl}$  and the resulting solution extracted with ether, which removed a material giving a green colour with  $\text{FeCl}_3$  solution. The acid liquid was neutralised, cleared with lead acetate and found to give an intense Molisch reaction and to reduce hot Fehlings' solution. The osazone was prepared by addition of 0.1 gm. phenylhydrazine hydrochloride, 0.2 gm. of sodium acetate and heating in the usual way. It was recrystallised from alcohol and had M.P.  $180^\circ$ .

Mixed with Rhamnoseosazone of M.P.  $181^{\circ}$  the mixture melted at  $180^{\circ}$ .

Microanalysis:

N.

Found 16.37.

Rhamnosephenylasazone  $C_{18}H_{22}O_3N_4$  requires 16.27.

The substance is therefore a glycoside of the methylpentose rhamnose. The aglycone was not further investigated.

#### MAIN FILTRATE FROM THE BASIC LEAD ACETATE PRECIPITATION.

This was concentrated, under reduced pressure, to 500 c.c. Of the pale yellow liquid, 10 c.c. killed a 2.3 kilo rabbit in three hours with symptoms of typical Gifblaar poisoning. A carbohydrate material was removed by the addition of 20 per cent. sodium hydroxide solution until further precipitation ceased. This occurred when the mixture was about normal in sodium hydroxide. The buff-coloured, somewhat gelatinous precipitate was centrifuged off and washed, then dissolved in warm, dilute sulphuric acid, using such a quantity that the final reaction was approximately neutral. A small quantity of insoluble material was filtered off and on the pale, yellow-brown solution certain qualitative tests performed as follows:—

##### *Tests upon Polysaccharide Material.*

Molisch test.....	+ intense.
Biuret test.....	-
Millon's test.....	-
Ferric chloride.....	faint green colour.
Fehling's solution.....	not reduced; reduced after acid hydrolysis.
Ammoniacal silver nitrate	reduced on warming.
Orcinol test .....	$\pm$
Naphthoresorcin test.....	+
Lime water.....	small white precipitate.

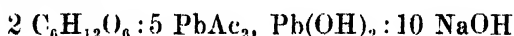
After hydrolysis by dilute sulphuric acid, a considerable quantity of calcium sulphate separated and the hydrolysate gave positive reactions for sugars. The polysaccharide material which was not soluble in absolute alcohol was not further investigated as tests proved it to be non-toxic. It is probably a complex of the uronic acid type.

To the alkaline main filtrate from the carbohydrate precipitate, sufficient acid was added to bring to neutrality. Since tests for free reducing sugars were strongly positive and these materials, as recorded by Green (see Steyn, 1928), always accompanied the toxic principle in every attempt he made to isolate it, it was decided to remove them completely at this stage of the general investigation.

The exact quantity of reducing sugar, calculated as glucose, was determined upon an aliquot of the main solution using Benedict's quantitative solution and his method of determination.

Thus, in one instance, 25 c.c. of reagent (=0.05 gm. Glucose) required 33.5 c.c. of 1 in 10 dilution of the main solution to effect complete reduction. Since the total volume was at this stage 750 c.c. there was therefore present 11.19 gm. of glucose in all.

For precipitation, basic lead acetate solution (accurately titrated by means of ammonium molybdate) and sodium hydroxide were added in the proportions—



that is to say, 156.6 c.c. of basic lead acetate solution containing 56.14 gm. basic salt per 100 c.c. and 12.43 gm. of NaOH dissolved in about 100 c.c. water.

The dense precipitate was removed by suction, the filtrate neutralised and excess of lead removed by hydrogen sulphide. The pale straw-coloured filtrate (1,020 c.c.) contained no reducing sugars but retained its original toxicity to rabbits.

Tests performed at this stage indicated the presence of basic materials which could be precipitated by mercuric chloride and phosphotungstic acid respectively.

Accordingly, the bulk of the liquid was reduced by vacuum distillation to 200 c.c. and a fair excess of saturated mercuric chloride solution added. After four days, the precipitate was removed and mercury eliminated from both fractions.

#### MERCURIC CHLORIDE PRECIPITATE.

The concentrated Hg-free solution of this fraction was very dark brown in colour and resisted attempts to decolorise it. Some tarry material was, however, removed and the following tests performed upon the filtrate:—

Biuret reaction.....	.
Millon reaction.....	-
Ninhydrine reaction.....	±
Diazobenzenesulphonic acid	++ intense red primary colour; after reduction, etc., yellow.
Phosphotungstic acid.....	± white ppt. soluble on heating but reappearing on cooling.

The reactions suggest histidine or a histidine-like substance. All attempts to obtain pure histidine hydrochloride from the mixture were, however, unsuccessful.

The main filtrate from the mercuric chloride precipitation was still toxic. Sulphuric acid was added to about 5 per cent. concentration and phosphotungstic acid added so long as a precipitate formed. A very large quantity was required. The bulky white precipitate was filtered off on a Jena glass filter, washed and decomposed in the usual way with hot baryta. The colourless liquid had the following characteristics.

SOLUTION OF BASIC FRACTION PRECIPITATED BY  
PHOSPHOTUNGSTIC ACID.

Diazobenzenesulphonic acid	-	
Wagner's reagent.....	+	Amorphous precipitate, with fine spear-like prisms appearing but collapsing to oily drops on addition of water.
Dragendorff's reagent....	+	
Aloxan reaction.....	++	red-violet changing to violet blue on addition of sodium hydroxide.
Solution boiled with NaOH		Alkaline, ammoniacal vapours evolved.

It was clear that a mixture of bases was present, amongst which choline was suspected. To a portion, evaporated to dryness and taken up in absolute alcohol, an alcoholic solution of mercuric chloride was added. The precipitate was centrifuged off, washed with 96 per cent. alcohol and decomposed. The solution was found to give the Florence reaction with Iodine in potassium iodide. On evaporation a hygroscopic crystalline residue was left—presumably choline hydrochloride. 0.10 gm. dissolved in 0.5 c.c. water was injected into the dorsal lymph sac of a frog (*Bufo regularis*). Within about ten minutes respiratory distress was noticed; the animal gradually lost all reflexes and died fifteen minutes after dosing.

1.3 gm. was given per os to a rabbit but no untoward symptoms were noticed.

Lack of material prevented a more rigorous identification, but all the evidence pointed to the identity of this base with *choline*.

To the remainder of the phosphotungstic acid fraction, auric chloride was added in excess, the precipitate removed and dissolved in warm water. On cooling, stellate aggregates of a crystalline gold salt separated. These were washed with water and alcohol and had M.P. 185°. On analysis they proved to be the basic gold salt of *trigonelline* which has M.P. 186°.

Microanalysis:

	C	H	N	Au.
Found.....	21.94	2.51	3.63	36.94
(C <sub>7</sub> H <sub>7</sub> O <sub>2</sub> N) <sub>4</sub> · 3 H <sub>2</sub> AuCl <sub>4</sub> requires	21.41	1.98	3.57	37.73

The normal gold salt was also prepared by recrystallisation in excess of acid auric chloride solution. It had M.P. 194°.

Trigonelline aurichloride melts at 197°.

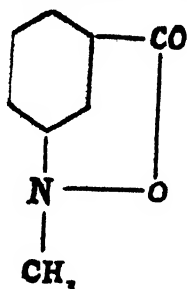
Microanalysis:

	An
Found.....	40.97
C <sub>7</sub> H <sub>7</sub> O <sub>2</sub> N <sub>4</sub> · H <sub>2</sub> AuCl <sub>4</sub> requires.....	41.31

In all about 0.2 gm. of gold salt was isolated.



The alkaloide trigonelline is widely distributed in the vegetable kingdom and is practically non-toxic. Its constitution is—



The main filtrate was freed from phosphotungstic acid by means of baryta and concentrated in vacuo to 200 c.c. It retained its original toxicity, the equivalent of 10 gms. of plant proving fatal to a rabbit.

On evaporation, a large amount of crystalline inorganic material separated. The mass was extracted by hot 98 per cent. alcohol which removed the toxin but eliminated a large quantity of inorganic salts. The presence of sodium acetate proved very troublesome but by repeated extractions, a solution was obtained, still highly toxic in doses of approximately 0.1 gm. per kilo and considerably purified as compared with the original plant extract. Certain tests were carried out to gain further information concerning the nature of the toxic principle.

#### TESTS UPON A HIGHLY PURIFIED CONCENTRATE.

<i>Test.</i>	<i>Conclusion.</i>
1. Basic lead acetate..... -	Toxin probably not acidic.
2. Phosphotungstic acid and all alkaloid reagents.... -	Toxin not basic in character.
3. Molisch ..... -	Toxin not carbohydrate.
4. Biuret..... -	No peptones, etc., present.
5. Ninhydrine..... + +	Presence of amino acids or a primary amino group in toxin.
6. Diazobenzene sulphonie acid..... -	Absence of phenolic substances.

The solid residue contained N (Lassaigne's test) but neither S nor halogens. The value of the positive Ninhydrine reaction was difficult to assess on account of the fact that any simple amino-acids derived from the proteins of the plant would have remained in the solution up to this stage. A portion of the residue was treated with nitrous acid, the solution boiled and subsequently tested for toxicity. It proved to be as active as before, from which it might be concluded that the ninhydrine reaction was given by amino-acid impurities or conversely that should a primary amino group be present in the toxin, this can be replaced by hydroxyl without influencing toxicity. In a latter experiment the absence of primary amino groups from the toxin was definitely proved.

### SPECIAL METHODS OF ATTEMPTED ISOLATION.

The essential difficulty underlying all attempts at isolation of the Gifblaar toxin is the circumstance that the active material is soluble only in water and it becomes extremely difficult therefore to remove other water-soluble substances such as simple amino-acids, inorganic salts, etc., which are also present in the plant extracts. It may be noted here that, in the present work, toxin concentrates have been obtained for the first time free from all carbohydrate material, by use of the technique described above. This removes all doubt about the possible carbohydrate nature of the toxin itself.

Another embarrassing property of the active material is its remarkable chemical inertness. No substance has been found which will form with it an insoluble compound, neither does it seem to contain any easily identifiable chemically reactive grouping. On account of these difficulties, various specialised methods of approach were investigated in the hope of achieving further purification.

### DIALYSIS AND HIGH-PRESSURE ULTRAFILTRATION.

An aqueous solution of a highly purified concentrate was dialysed in a collodion bag (prepared from specially prepared gun-cotton solution) against distilled water. Animal tests showed that the toxin dialysed readily into the surrounding medium.

Attempts were then made to use membranes of very low permeability, employing a steel filtration unit subjected to high pressures of nitrogen gas in order to hasten filtration. Mc.Bain has been able, by similar means, to effect a partial filtration of sucrose molecules from aqueous solutions, so it was hoped that an enrichment, at least, of the toxin would be accomplished. Even with pressures up to 80 atmospheres used in conjunction with the densest filters preparable, no appreciable retention of the toxin occurred. It must therefore be of comparatively small molecular dimensions.

### HIGH VACUUM DISTILLATION.

This method is one frequently employed in the purification of materials which do not crystallise readily and which are fairly resistant to heat. The vacuum was obtained by a small Pfeiffer pump. All glass parts of the apparatus were thoroughly dried out before commencing the experiment. About 20 gms. of material was placed in the distilling flask and the following fractions collected.

	<i>Oil bath temperature.</i>	<i>Vapour temperature.</i>	<i>Pressure.</i>	<i>Appearance of fraction.</i>
Fraction I....	90-110°	44-49°	3 m.m.	Water-clear liquid, acid in reaction.
Fraction II....	120-200°	50-125°	3 m.m.	Pale yellow oil in small quantities, slightly acid.
Fraction III....	216-275°	130-4°	3-4 m.m.	Yellow-brown viscous oil, pungent smell, alkaline, not completely soluble in water.

**Flask residue.**—Carbonaceous mass smelling strongly of ammonia-like bases. Only a negligible quantity soluble in water.

Each fraction was dosed to a test rabbit. The only animal showing any symptoms was that which received fraction III. It lost appetite, was somewhat drowsy and died four days after dosing. The symptoms did not suggest Gifblaar poisoning.

Clearly, then, vacuum distillation and sublimation are of no avail.

#### CONTINUOUS BUTYL ALCOHOL EXTRACTION.

Owing to the strongly positive ninhydrine reaction given by the purest concentrates it was thought possible that the toxin might be a substance of the amino acid or peptone type. Accordingly the continuous butyl alcohol extraction technique of Dakin was tried. A purified concentrate was placed in a Kutscher-Stendel apparatus and the extraction maintained for six days. A deposit of solid material formed in the butyl alcohol extract which was nearly black in colour. After removing butyl alcohol the fractions were tested out upon rabbits as follows:—

M.L.D. of original concentrate  $\pm 5$  c.c.

M.L.D. residual liquid after extraction  $\pm 6.5$  c.c.

Butyl alcohol extract contained about 3 M.L.D.'s.

Apparently butyl alcohol removes only traces of the active principle. This result was especially disappointing since Dakin showed that the simple amino-acids, though practically insoluble in butyl alcohol, could be extracted quantitatively from aqueous solutions by use of this solvent, no doubt owing to the more ready solubility of some modification of the molecule, like the "Zwitterion", which reforms as soon as that originally present in minute quantity has been taken up by the ascending bubbles of the alcohol.

Numerous other methods of isolation were tried but since none proved successful the detailed description of each will be omitted.

It was thought that possibly some derivative might be formed by taking advantage of the reactivity of a group resident in the toxin molecule and inducing combination with a suitable reagent. Such a derivative might reasonably be expected to possess solubilities different from the parent molecule and hence offer a chance of isolation in the pure form. Of such groupings, those most likely to be present in a natural product are  $>CO$ ,  $-OH$  and  $>NH$ .

#### TEST FOR $>CO$ GROUPS.

To 10 c.c. of a purified concentrate (MLD  $\pm 10$  c.c.), was added sufficient hydrochloric acid to render the solution 2 normal and then 8 c.c. of Brady's reagent (0.5 gm. 2:4 dinitrophenylhydrazine in 30 c.c. of warm 2 normal hydrochloric acid). A brick-red precipitate formed which was centrifuged off and washed well with dilute acid and then water. From the mother liquor, excess of reagent was removed by repeated shaking with chloroform, finally from the neutralised solution. This liquid was then administered to a 2.3 kilo rabbit. The animal died, indicating that the toxic principle had not been removed. It is safe to conclude therefore that in all probability no  $>CO$  or  $-CHO$  group is present in the toxin molecule.

TEST FOR PRESENCE OF  $-OH$  OR  $>NH$  GROUPS.

Both the hydroxyl  $-OH$  and imino  $>NH$  groups are capable of being benzoylated by means of benzoyl chloride and the derivatives formed have usually lesser solubility in water and greater solubility in organic solvents than have the parent molecules.

Accordingly, 20 c.c. of the same toxic preparation as was used in the previous experiment ( $\approx 2$  M.L.D.) was benzoylated with benzoyl chloride and sodium hydroxide by the Schotten-Baumann method. A certain amount of a brownish insoluble material separated out during the course of the benzoylation. The alkaline liquid was shaken repeatedly with ether. It was then acidified and again ether extracted. Both extracts and the residual solution were dosed separately to rabbits. The animal receiving the residual solution died in  $4\frac{1}{2}$  hours from typical Gifblaar poisoning. There is thus no ether-soluble benzoyl derivative formed and should the toxin molecule possess either an  $-OH$  or  $>NH$  group one is forced to conclude that this grouping is in no way essential to the manifestation of toxic activity. It seems more probable that these groups are absent from the toxin.

To summarize the evidence available at this point it would appear that the molecule of the Gifblaar toxin contains neither  $-COOH$ ,  $-OH$ ,  $>NH$ ,  $-NH_2$  nor  $>C=O$  groups. It possesses neither acidic nor basic character and appears to be chemically very inert. One is forced to the conclusion that it must be a very peculiarly constructed molecule. The only possibility that to the writer seems open is that it must be a tertiary amine, the grouping  $>N$  having in this case exceedingly feebly basic or neutral characteristics. Unfortunately no possible method of isolation is suggested by this consideration, tertiary bases being comparatively non-reactive.

## EXPERIMENTS UPON THE STABILITY OF THE TOXIN TO VARIOUS AGENTS.

Certain evidence has already been presented, demonstrating that the Gifblaar toxin is thermostable and not readily attacked by acids or alkalis. In order to gain precise information, the following experiments were carried out:—

A concentrate was prepared of which the M.L.D. for rabbits was found to be  $\pm 5$  c.c. Individual 5 c.c. portions were then treated as follows:—

- (a) Acid potassium permanganate (0.01 N) solution added so long as decolorisation occurred at room temperature: 30 c.c. required in all. The mixture was neutralised and dosed.
- (b) 2 c.c. of perhydrol added. After one hour at room temperature the mixture was dosed.
- (c) 1.5 c.c. normal sodium hydroxide added (i.e. to 1 per cent.). Mixture placed in boiling water bath for 1 hour, cooled, neutralised and dosed.
- (d) 1.2 c.c. normal sulphuric acid added. Treated as in (c).

All four rabbits receiving these preparations died. It is clear therefore that the toxin is stable to mild oxidising agents and not altered appreciably by hot 1 per cent. acid or alkali; it is not therefore a substance suffering hydrolytic fusion.

#### ELIMINATION OF AMINO-ACIDS AND ABSENCE OF A PRIMARY AMINO GROUP.

Since the most carefully purified extracts always gave a strongly positive ninhydrine reaction, suspicion was entertained that the active principle might contain a primary amino group although the experiments recorded on pages ..... and ..... rendered this somewhat unlikely. Amino acids may be precipitated from complex mixtures by the use of mercuric acetate and sodium carbonate and it was therefore decided to subject a concentrate to this procedure and ascertain in which fraction the toxicity remained.

To 25 c.c. of the same solution as used above (M.L.D.  $\pm 5$  c.c.) were added 10 per cent. sodium carbonate and 25 per cent. mercuric acetate solutions until precipitation of mercury compounds was completed as shown by the orange-yellow colour of the final turbidity.

The precipitate was removed, suspended in dilute hydrochloric acid and gassed by hydrogen sulphide; mercury was also removed from the filtrate.

One rabbit was given  $2/5$  of the precipitate fraction, corresponding to 2 M.L.D., but suffered no ill effects. Another, receiving  $3/10$  of the filtrate ( $\approx 1\frac{1}{2}$  M.L.D.) died  $3\frac{1}{2}$  hours after dosing. The toxin is therefore not precipitated by the reagent.

The active filtrate fraction was pale yellow in colour but gave no reaction for amino groups with ninhydrine thus proving that primary amino groups  $-NH_2$  are absent from the toxin.

A one M.L.D. equivalent of this amino-acid free solution was evaporated to dryness. The weighed residue, a pale yellow viscous syrup, was ashed and the weight of the ash subtracted thus affording the weight of organic matter present in a single lethal dose. A total nitrogen kjeldahl determination was carried out upon a second M.L.D. equivalent. The results were as follows:—

Weight of organic matter	= 0.5832 gm.
Total N.....	= 21.4 mgm.

This preparation had not been subjected to such a rigorous series of purifications as some of those formerly employed but especial care had been taken to eliminate all nitrogen-containing impurities such as proteins, amino-acids, ammonium salts, etc. The comparatively high nitrogen content of the residue, amounting to 3.7 per cent., would appear to offer evidence strongly presumptive of the presence of nitrogen as an integral constituent of the molecule of the toxin. It is of interest, in this connection, to record that the fumes evolved on ashing the material were found to give a fairly strong pine-splinter test. The formation of pyrrol by pyrolysis is of course no specific

indication of the presence of pyrrollic derivatives in the material but indicates nevertheless that organic, nitrogenous compounds are present.

It is highly probable that the Gifblaar toxin contains cyclically bound nitrogen.

#### RAPID METHOD FOR THE PREPARATION OF CONCENTRATES.

The method of purification outlined is very laborious and time-consuming. Two circumstances, in particular, render the procedure troublesome, namely the large amount of certain reagents required and the great mass of inorganic material which accumulates in the final solutions.

In order to obviate these difficulties and provide a rapid method for obtaining a fairly pure concentrate the following method was devised. A watery extract of the plant was cleared with basic lead acetate and the lead-free filtrate concentrated in vacuo, *without neutralisation*, to a small bulk. Drying was continued in front of a fan at room temperature. The syrupy material was then stirred repeatedly with acetone which removed the last traces of acetic acid and served to dehydrate the material. It was then taken up in a suitably large volume of 96 per cent. alcohol rejecting the material remaining undissolved. An equal volume of absolute alcohol was then added and the precipitate discarded. The remaining 98 per cent. alcohol solution contains nearly all of the active principle originally present and in a condition sufficiently pure to serve as the starting point for further experiments. It was found by varying the procedure and extracting the dehydrated mass with absolute alcohol before dissolving in 96 or 97 per cent. that a small quantity of additional inert material could be removed.

The detailed procedure and results obtained in this series of investigations is best illustrated by the following charts:—

#### CHART I.

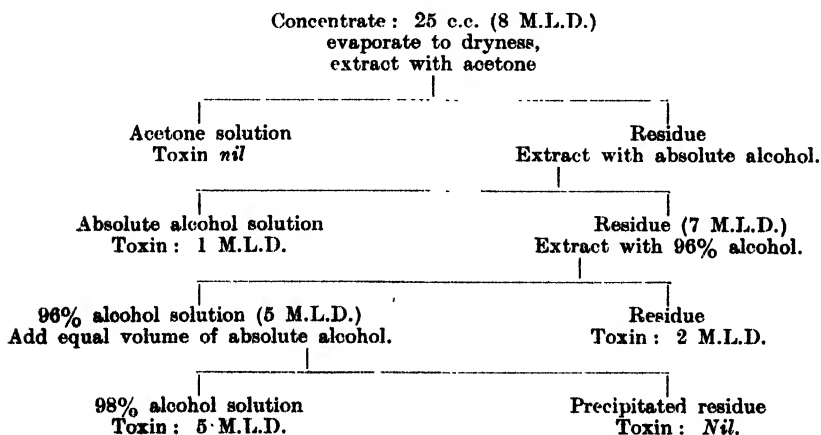
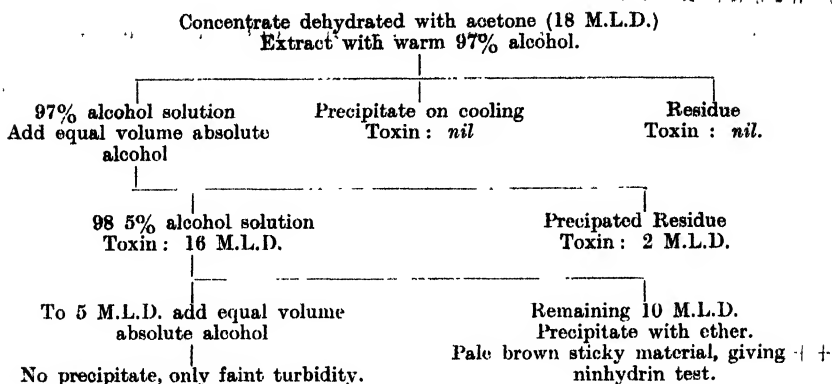


CHART II.



DISCUSSION AND SUMMARY.

Although the investigations recorded in this paper have not resulted in the isolation of the toxic principle of the Gifblaar, *Dichapetalum cymosum* in a state of chemical purity, it is felt that certain valuable information has been obtained relative to the nature of this toxin. In addition, some interesting substances have been isolated from the plant in the course of chemical manipulations. These include :

- (1) A catechol tannin.
- (2) A yellow colouring matter, shown to be a methylpentoside, yielding rhamnose on hydrolysis.
- (3) A histidine-like base.
- (4) A base probably identical with choline.
- (5) The alkaloid trigonelline, the methyl-betaine of nicotinic acid.

Various specialised methods were tried for the purification of the toxin, including :

- (1) Dialysis.
- (2) Ultrafiltration under pressures up to 80 atmospheres of nitrogen.
- (3) High-vacuum distillation and sublimation.
- (4) Continuous extraction with butyl alcohol, etc.

Certain other chemical operations led to the conclusion that the following groups are definitely absent from the toxin molecule:—

Carboxyl ... ..	- COOH
Ketonic ... ..	>CO
Aldehydic ... ..	- CHO
Hydroxyl ... ..	- OH
Amino ... ..	- NH <sub>2</sub>
Imino ... ..	>NH

The active principle almost certainly contains nitrogen and it is suggested that this may be included in some cyclic structure. It may possibly occur as in tertiary bases  $\gg N$  but the molecule, as a whole, does not evince basic characters. Preparations, still highly toxic to rabbits, have been prepared containing no protein, amino-acid, or carbohydrate material, impurities which had not before been successfully removed. The chemical stability or inertness of the toxic substance is very remarkable in view of its pronounced physiological action. Thus, it resists boiling for one hour with 1 per cent. sulphuric acid or 1 per cent. sodium hydroxide solutions; it is not oxidised by potassium permanganate or by hydrogen peroxide in the cold and it is thermostable.

*In view of this stability and the absence of any substance known to combine with or precipitate the toxin, the hope of finding any specific prophylactic or curative substance for use in Gifblaar poisoning becomes very remote indeed.*

#### ACKNOWLEDGMENT.

I wish to thank Dr. D. G. Steyn for his unfailing interest throughout the course of these investigations.

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## **Hydrocyanic Acid in Grasses.**

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- I. INTRODUCTION.
- II. A LIST OF TOXIC GRASSES.
- III. THE METHODS OF EXTRACTION.
- IV. PRUSSIC ACID CONTENT AND THE LETHAL DOSE.
- V. CONDITIONS IN THE ANIMAL FAVOURING OR PREVENTING TOXICITY.
- VI. EXTERNAL CONDITIONS LEADING TO TOXICITY OF THE PLANT.
  - (a) Climate and soil.
  - (b) Diurnal and seasonal variations.
  - (c) Wilting.
- VII. INTERNAL CONDITIONS OF THE PLANT LEADING TO TOXICITY.
  - (a) Theory of Goris.
  - (b) Theory of Gautier.
  - (c) Theory of Meyer and Schulze.
  - (d) Theory of Bach.
  - (e) Theory of Treub.
  - (f) Objections to the theory of Treub.
  - (g) Facts in favour of the theory of Treub.
- VIII. EFFECT OF FREE PRUSSIC ACID ON THE PLANT.
- IX. PRUSSIC ACID IN GLYCOSIDIC OR NON-GLYCOSIDIC FORM.
- X. PRUSSIC ACID AS AN ORGANIC COMPOUND.
- XI. EXPERIMENTS :—
  - (a) Experiments on *Sorghum verticilliflorum*.
  - (b) Experiments on *Eustachys paspaloides*.
  - (c) Extractions.

### **I. INTRODUCTION.**

The question of prussic acid in grasses is of practical as well as theoretical importance. The numerous deaths in animals caused by toxic fodder raise the subject to a vital issue for farmers. From a theoretical point of view the problem throws much light on the

physiology of the plant. Indeed, in many cases it distinctly shows the influence of environment on metabolism and also provides some hints on the possible intermediate products which could arise in the synthesis or disintegration of protoplasm. We are probably touching here on some fundamental activity of the living cell, which may enlighten us one day on the reactions leading to protein synthesis.

As in most cases of agricultural research, the theoretical aspect of the question provides the solution to the practical one. Many of the experiments mentioned in this paper have, however, been made only with the view of obtaining immediate useful conclusions for the farmer. It is perhaps this lack of academic interest in the problem which leaves us so much in the dark with respect to conditions leading to the production of prussic acid in grasses.

The publications on the subject are already abundant; a summary and a synthesis are therefore urgently called for, especially in the presence of numerous conflicting observations, which reveal once more how involved the response of the plant is towards its environment.

In our endeavour to understand some of the drastic changes observed in plants, we also far too often look for outstanding causes. Yet many of the remarkable fluctuations in prussic acid content are produced only by minute variations of climate and chemical composition of the soil. An experimental method to record such oligodynamic changes of environment is badly needed and were such a method at hand we would certainly see clearer in the problem under discussion.

In order to discuss our subject from all possible angles we shall also have to deal with a series of theories, not directly connected with grasses, the conclusion of which may however have a bearing on the plants we are dealing with. The aim of the paper is therefore not merely a compilation, but a synthesis of the existing knowledge on the question of prussic acid in grasses.

## II. A LIST OF TOXIC GRASSES.\*

Prussic acid occurs in grasses either free or in the nitrile form linked to a sugar and benzaldehyde-like substances. It is doubtful whether they all contain the same glycoside; acetonecyanhydrin has however not as yet been detected in them. In most cases the plant possesses the ferment necessary to split its glycoside; sometimes the former may occur alone. No grasses are as yet known where the glycoside alone is present, yet in future such cases may still be found, if we submit the Gramineae to a severe test.

In the following list, the authors in brackets are those who have been dealing with the plant, or at least have quoted it. The first name is not necessarily that of the author who made the first investigation, because it is often difficult, if not impossible, to determine

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\* I am very much indebted to Professor A. S. Hitchcock, National Herbarium, Washington, who, through the courtesy of Dr. E. P. Phillips, has supplied me with the most recent nomenclature of the grasses mentioned in this list. I also wish to thank Miss L. Chippendal, of the National Herbarium, Pretoria for her assistance.

who started the original research. When the information is available (only from Petrie) we shall state whether the plant contains glycoside+ferment, or ferment alone.

1. *Andropogon intermedius* R. Br., native in N.S. Wales, (Petrie 1913), glycoside+ferment, strong reaction in summer.
2. *Andropogon ischaemum* Linn. Wide distribution. (Petrie 1913), glycoside+ferment; strong reaction in the summer of N.S. Wales.
3. *Andropogon micranthus* Kunth., native in N.S. Wales, (scented grass) (Petrie 1913), glycoside+ferment, but only trace, sometimes entirely free.
4. *Amisopogon arenaceus* R. Br., native in N.S. Wales. (Petrie 1913).
5. *Antheophora pubescens* Nees. South Africa. Henrici 1926, Steyn 1934); contains large amounts of prussic acid in the wilted state.
6. *Anthoranthum odoratum* Linn. World wide. (Petrie 1913), ferment only.
7. *Aristida congesta* R. & S. South Africa. (Henrici 1926, Steyn 1934) yields prussic acid especially when the leaves are rolled.
8. *Aristida uniplumis* Licht. South Africa. (Henrici 1926, Steyn 1934), yields prussic acid when wilted.
9. *Arundo conspicua* Forsk. (*Cortaderia conspicua*) (Greshoff 1909, Pammel 1911). There is no record of this species ever having been transferred to *Cortaderia* under which genus it is quoted by Greshoff and Pammel.
10. *Axonopus compressus* Beauv. (*Paspalum platycaulon* Poir.) N.S. Wales. (Petrie 1913), ferment only, during part of the year.
11. *Bambusa arundinacea* Willd. Wide distribution. (Walter, Krassnosselska, Maximov and Maltschewsky 1911, Petrie 1913, Wehmer 1929).
12. *Bouteloua gracilis* (H. B. & K.) Lag. (*Bouteloua oligostachya* Torr.). Introduced from Mexico into N.S. Wales. (Petrie 1913), glycoside+ferment, maximum in mid-summer in N.S. Wales.
13. *Briza minor* Linn. World wide distribution. (Couperot 1908, Greshoff 1909, Pammel 1911, Petrie 1913). Petrie's investigations in 1913 show negative results throughout the year.
14. *Catabrosa aquatica* (L.) Beauv. (Petrie gives 1908 as first date of record, but no author.) (Greshoff 1909, Pammel 1911, Petrie 1913).

15. *Chaetium bromoides* (Prest.) Benth. (Petrie 1913), ferment only.
16. *Chloris polydactyla* (L.) Sw., introduced from South America to N.S. Wales. (Petrie 1913), glycoside + ferment, throughout the year.
17. *Chloris truncata*, R.Br. (star grass), native in N.S. Wales, (Petrie 1913), glycoside + ferment, strong reaction only in January.
18. *Chloris ventricosa*, R.Br., native in N.S. Wales. (Petrie 1913), glycoside + ferment, strong reaction only in January.
19. *Cortaderia argentea* Stapf. var. *gigantea*, *rosea* and *variegata*. No record of the publication of the varieties could be found. South American Pampas grass, cultivated in N.S. Wales. (Petrie mentions 1906 as year of first record, but no author.)  
(Petrie 1913), glycoside + ferment, all varieties show strong reactions the whole year through.
20. *Cortaderia kermesiana*. (Greshoff 1909, Pammel 1911). There is no record of such a species.
21. *Cynodon bradleyi* Stent. Wide distribution. Cultivated as a lawn grass. (Steyn 1929 and 1934).
22. *Cynodon dactylon* (L.) Pers. Wide distribution. (Petrie 1913, found ferment only; Wehmer 1929, Steyn 1934).
23. *Cynodon incompletus* Nees. South Africa, N.S. Wales, (Maiden 1912, Schimmel 1913, Petrie 1913, Wehmer 1929, Steyn 1934). According to Petrie it was still doubtful in 1913 whether this grass had been introduced from South Africa, or is indigenous in Australia. According to the same author the grass contains glycoside + ferment, shows a strong reaction in winter only, loses its glycoside through desiccation while the ferment remains.
24. *Cynodon transvaalensis*, Burtt Davy. Transvaal. (Steyn 1934). Wilted specimens contain large amounts of prussic acid. (See also under climate and soil.)
25. *Danthonia semianularis* (Labill.) R.Br., native in N.S. Wales. (Petrie 1913), glycoside + ferment, faint reaction.
26. *Digitaria eriantha* Steud. South Africa (Henrici 1926, Steyn 1934), prussic acid in wilted specimens.
27. *Dactyloctenium aegyptium* (L.) Richt. (*Eleusine aegyptiaca* Pers.). South Africa, N.S. Wales. (Petrie 1913), glycoside + ferment, reaction during part of the year only.
28. *Eleusine coracana* Gaertn. (Raybaud 1913).
29. *Eleusine indica* Gaertn. South Africa, N.S. Wales. (Petrie 1913, Raybaud 1913), glycoside + ferment, reaction during part of the year.

30. *Eragrostis pilosa* Beauv. (Petrie 1913), ferment only, during part of the year.
31. *Eragrostis pectinacea* Michx. (*Eragrostis purchii* Schrad.) (Petrie 1913), ferment only, during part of the year.
32. *Eustachys paspaloides* (Vahl) Lanza et Matti. Wide distribution. (Petrie 1913, Rosenthaler 1925, Henrici 1926, Wehmer 1929, Steyn 1934). According to Petrie, glycoside + ferment, in N.S. Wales, very strong reaction in November, faint or nil during the rest of the year.
33. *Elymus* sp. (Greshoff 1909, Pammel 1911).
34. *Echinochloa colona* Link (*Panicum colonum* L.) (Petrie 1913), ferment only, during part of the year.
35. *Festuca lachenalii* Sparm. (*Festuca poa* Kunth) (Petrie mentions first record 1908, but no author). (Greshoff 1909, Pammel 1911, Petrie 1913).
36. *Glyceria aquatica* (L.) Wahl. (*Poa aquatica* L.) (Jorisson 1884, Greshoff 1906, 1909, Pammel 1911, Petrie 1913, Guérin 1932).
37. *Glyceria canadensis* (Michx.) Trin. (*Panicularia canadensis* Michx.) Kuntze, North America (Alsberg and Black 1915).
38. *Glyceria grandis* Wats. (*Panicularia grandis* Nash), North America (Alsberg and Black 1915).
39. *Glyceria nervata* (Willd.) Trin. (*Panicularia nervata*) (Willd) Kuntze, North America (Alsberg and Black 1915).
40. *Heemarthria compressa* R.Br. (Petrie 1913), ferment only, during part of the year.
41. *Holcus lanatus* Linn. Wide distribution (Petrie mentions date of first record 1908, but not author). (Greshoff 1909, Pammel 1911, Petrie 1913).
42. *Lagurus oratus* Linn. South Africa, N.S. Wales. (Petrie 1913), ferment only, during part of the year.
43. *Lamarckia aurea* Moench. (Couperot 1908, Greshoff 1909, Petrie 1913). Petrie has found no trace of prussic acid in his specimens in N.S. Wales.
44. *Leptochloa decipiens* (R.Br.) Druce (Bentham refers this sp. to *L. chinensis* Nees). N.S. Wales. (Petrie 1913), glycoside + ferment, strong production of HCN throughout the year.
45. *Leptochloa dubia* (H.B. & K.) Nees (*Diplachne dubia* Scribn.) A Mexican grass introduced into N.S. Wales. (Petrie 1913), glycoside + ferment, one of the grasses that yields the highest amount of prussic acid tested by Petrie. Evolves constantly free acid throughout the year.
46. *Lolium Lamarckii* L. (Cornevin 1893, Greshoff 1909, Pammel 1911). No such species is on record.

47. *Lolium perenne* L. (Miquel 1838, Cornevin 1893, Greshoff 1909, Pammel 1911).
48. *Melica altissima* L.      )
49. *Melica ciliata* L.        )
50. *Melica nutans* L.        )
51. *Melica uniflora* Retz.    )
- (Fitschy 1906. Petrie 1913. Ray-  
                                    baud 1913, Wehmer 1929) (Gres-  
                                    hoff 1909 and Pammel 1911 mention  
                                    genus *Melica*).
52. *Melica magnolii* Gren. et Godr. (Mirande 1909, Raybaud 1913).
53. *Panicum bulbosum* H. B. & K. (Petrie 1913), ferment only, during part of the year.
54. *Panicum divaricatissimum* R.Br. var. *normale* Benth. (Petrie 1913), ferment only, during part of the year.
55. *Panicum junceum* Nees. (Greshoff according to Pammel 1911).
56. *Panicum maximum* Jacq. Wide distribution. (Brünnich 1903, Greshoff 1906, Petrie 1913). Petrie has not found any prussic acid. Contains dhurrin according to Hadders and Wehmer in Klein's Handb. der Pflanzenanalyse 1932.
57. *Panicum muticum* Forsk. (Brünnich 1903, Greshoff 1906, Petrie 1913). Petrie has not found any prussic acid. Contains dhurrin according to Hadders and Wehmer in Klein's Handbuch der Pflanzenanalyse 1932.
58. *Panicum strictum* R.Br. (Bentham names this *P. marginatum* var. *strictum* Benth.) (Petrie 1913), ferment only, during part of the year.  
                                     *Panicum* as a genus was mentioned by Greshoff in 1909 and Pammel in 1911.
59. *Paspalum scrobiculatum* Linn. South Africa, N.S. Wales. (Petrie 1913), ferment only, during part of the year.
60. *Pennisetum latifolium* Spreng. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
61. *Phalaris coerulescens* Desf. (*Phalaris aquatica* L.) (Jorisson 1885. Jorisson and Hairs 1891, Wehmer 1929).
62. *Poa nemoralis* L. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
63. *Pogonarthria squarrosa* (Licht.) Pilger. South Africa. Henrici 1926, Steyn 1934). Wilted specimens contain prussic acid.
64. *Rhaphis gryllus* (L.) Desv. (*Andropogon gryllus* L.) Native in N.S. Wales. (Petrie 1913), glycoside + ferment, only a trace and only in winter.
65. *Rhaphis montana* (Stapf.) Phill. (*Chrysopogon serrulatus* Trin.) South Africa. (Henrici 1926).

66. *Sorghum halepense* (L.) Pers. [*Andropogon halepensis* (L.) Brot.]. Petrie indicates first record as 1903, but does not mention the author.) (Pammel 1911, Petrie 1913).
67. *Sorghum halepense* (L.) Pers. forma (*Andropogon halepensis* Sibth var. *mutica* Hack.) Native in N.S. Wales (Petrie 1913), glycoside + ferment.
68. *Sorghum verticilliflorum* Stapf. Johnson Grass. (Crawford 1906, Steyn 1934).
69. *Sorghum vulgare* Pers. [*Andropogon sorghum* (L.) Brot.] (Cornevin 1838, Abbot 1887, Palmeri 1887, Dunstan and Henry 1902, Balfour 1903, Peters, Slade and Avery 1903, Ravenna et Zamorani 1903, Ravenna et Peli 1907, Greshoff 1909, Pammel 1911, Petrie 1913, Wehmer 1929, Steyn 1934). There is a remarkable variation of the ferment. Certain leaves contain the ferment only (Petrie 1913).
70. *Sorghum vulgare* Pers. forma (*Sorghum nigrum* R. & S.) (Petrie 1913, Rosenthaler 1922, 1923, Wehmer 1929).
71. *Sorghum vulgare* Pers. forma (*Sorghum saccharatum* Pers.) (Petrie indicates first record in 1903, but does not mention the author). [Petrie 1913, Hiltner (date?), Wehmer 1929, Steyn 1934.] Raybaud has examined 26 Sorghums, but does not mention the species.
72. *Sporobolus fimbriatus* Nees. South Africa. (Henrici 1926, Steyn 1934), prussic acid in the wilted plant.
73. *Sporobolus virginicus* Kunth. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
74. *Stipa capillata* L. (Greshoff 1909, Petrie 1913).
75. *Stipa elegantissima* Labill. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
76. *Stipa gigantea*. (Petrie 1913, mentions year of first record 1906, but no author.) There is *S. gigantea* Link and *S. gigantea* Lag. It is impossible to decide which one was meant.
77. *Stipa hystericina* Spreng. (Hébert-Hein 104, Greshoff 1906, Petrie 1913).
78. *Stipa leptostachya* Griseb. (Hébert-Hein 1904, Greshoff 1906, Petrie 1913).
79. *Stipa Lessingiana*. Trin. & Rupr. (Greshoff 1909, Petrie 1913).
80. *Stipa tenuissima* Trin. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
81. *Stipa tortilis*. Desv. (Petrie mentions the year of record 1906, but no author) (Petrie 1913).



82. *Stipa verticillata* Trin. (Bentham refers this sp. to *S. micrantha* R.Br.) N.S. Wales. (Petrie 1913), ferment only, during part of the year.
83. *Themeda triandra* Forsk. South Africa. (Henrici 1916, Steyn 1934).
84. *Trichachne insularis* Nees (*Panicum leucophaeum* H. B. & K.) (Petrie 1913), ferment only, during part of the year.
85. *Triodia flava* (L.) (Smyth [*Tridens flavus* (L.) Hitchc.] (Alsberg & Black 1915).
86. *Uniola latifolia* Mich. N.S. Wales. (Petrie 1913), ferment only, during part of the year.
87. *Zea Mays* Linn. (Cornevin 1838, Greshoff 1909, Walsh 1909, male inflorescence; Pammel 1911, Burt Davy 1912, Petrie 1913, Rosenthaler 1913, pistils; Wehmer 1929, Steyn 1934).
88. *Zysia pungens* Willd. N.S. Wales. (Petrie 1913), ferment only, during part of the year.

### III. THE METHODS OF EXTRACTION.

It has been abundantly demonstrated that the prussic acid content of a grass varies considerably according to climate and season. We should therefore not be surprised to find a large discrepancy in the figures obtained by quantitative experiments. Some of these measurements are however open to criticism because they were not all carried out with a maximum care to avoid losses. Some preliminary tests should always be made to ensure that during the chosen process of extraction no acid or glycoside is eliminated.

The difficulties attached to quantitative determinations are shown by the following instance. Dowell (1919) has observed that three-quarters of the prussic acid disappears when the grass is submitted to desiccation. Petrie (1913) has discovered on *Cynodon incompletus* that while the plant is drying, the glycoside content gradually decreases while the amount of enzyme remains unchanged. The writer has however dried some grasses under controlled conditions and has found that during the process practically no acid escaped.

The above apparent contradiction may be an indication, not of elimination but of a transformation into other substances. In such a case drying would not show a discoloration of the Guignard paper, while the glycoside content may have diminished to a considerable extent. In future such possibilities will have to be borne in mind, when extractions are made.

Many authors have macerated their material in water. There is no doubt that this method involves a certain loss through two channels. The dying cells will allow a diffusion of the ferment and prussic acid is emitted as such. Moreover an aqueous solution of the acid is unstable and leads to formate of ammonia.

Bishop (1927) has submitted some of the current practices to a test and finds that distillation leads to untrustworthy results. The alcohol extraction is considered to be sound for the estimation of cyanogenetic glycosides in leaves. See also Narasimha Acharya (1933).

The following points should always be taken into account when a quantitative test is decided upon:—

1. HCN may be present in a glycosidic or non-glycosidic form.
2. During maceration the ferment may be liberating HCN.
3. The radical CN may be present in another combination from which it is not liberated by our methods of extraction.
4. Boiling the acid and its salts in water, or keeping it too long in an aqueous solution may cause loss by transformation into other substances.
5. The method may only liberate part of the prussic acid, the rest being retained by catalytic or steric hindrance.
6. The process of extraction may be creating HCN *de novo* from nitrogen compounds in the plant.

Rosenthaler (1932), in Klein's *Handbuch der Pflanzenanalyse* gives an extensive account of the best methods for determining prussic acid in plants and of extracting the glycoside. We need therefore not enlarge on the subject, except for the warning that each species of grass needs its own methods of extraction, which must be determined by preliminary experiments.

I wish, however, to call back to remembrance Greshoff's micro method (1889) for detecting prussic acid in plant tissues:—

“Place a freshly cut section not too thin and containing at least one layer of intact cells, in a 5 per cent. alcoholic potash solution; then transfer it after 15-90 seconds to a warm (60° C.) ferrous-ferric solution (2.5 per cent. ferrous sulphate + 1 per cent. ferric chloride) and leave it there for ten minutes and finally place it for from five to fifteen minutes in dilute hydrochloric acid (one part of conc. acid and six parts of water). A section so prepared shows minute agglomerations of Prussian blue wherever prussic acid occurred in the original section.”

This method may prove useful in many instances, as a side test in doubtful cases.

#### IV. THE PRUSSIC ACID CONTENT AND THE LETHAL DOSE.

There are comparatively few experiments in grasses which have been properly conducted to give a safe indication of their toxicity.

Hindmarsh (1930) has found in administering “Scheele's acid” (HCN) that the lethal dose per 1 lb. body weight of sheep and cattle is 1 mg. Avery (1903) has found that 0.4 gr. HCN *per os* will render a heifer very ill, but allow recovery. He does not state the weight of the animal. Steyn (1934) has computed from several authors (among which also Hindmarsh) that the figure for cattle is 2.2 mg. HCN per Kg body-weight intraperitoneally, and for sheep 2.2 mg *per os*.

Are we entitled, on the basis of these tests obtained by pure chemicals, to calculate the lethal dose in terms of so and so much grass, assuming we know the prussic acid content of the plant? Considering one of the experiments by Seddon and King (1930) we would feel inclined to answer in the affirmative. These authors have shown that in feeding *Acacia glaucescens* (containing sambunigrin) they confirm Hindmarsh's determination of 1 mg. per lb body-weight for sheep.

But curiously enough when feeding *pure* sambunigrin from *Acacia glaucescens*, the dose in terms of prussic acid was 2 mg. per lb. body-weight.

Petrie (1913) reports on an experiment made with *Cynodon incompletus* which was fed to sheep. The material contained 0.016 per cent. prussic acid. The lethal dose per sheep of 150 lb. was 2 lb. of grass which could liberate 0.14 grams of prussic acid. This confirms again the figure established by Hindmarsh as roughly 1 mg. per lb. body-weight.

Peters (1903) relates the case of a heifer which dropped to the ground ten minutes after having been driven into a Sorghum field. The animal was finally killed because it was obvious that it would not recover. The post-mortem showed 1½ lb. of sorghum in the paunch.

To arrive at an idea what the quantity of prussic acid involved in the last case may be, consider the maximum quantities of this substance extracted from grasses by the following authors:—

	<i>Per cent.</i>
Dowell (1919) highest per cent. obtained on <i>Andropogon Sorghum</i> ... ..	0.0514
Swanson (1921) highest per cent. obtained on Sudan grass ... ..	0.015
Avery (1903) highest per cent. obtained on <i>Sorghum vulgare</i> ... ..	0.014
Pinckney (1924) highest per cent. obtained on Sorghum grown on Coloma sand with 502 lb. nitrate per acre ... ..	0.136
Willaman and West (1915) highest per cent. obtained on Sorghum (Minnesota) ... ..	0.114

In assuming that the animal in Peters' experiment had taken Sorghum of the highest toxicity such as Pinckney had obtained, the amount of prussic acid present in the animal through the ingestion of 1½ lb. of grass would be about 0.8 gr. Assuming the animal to be about 200 lb., this would be four times the lethal dose.

But the important question here is not how much have we *introduced*, but how much can be *liberated* in such a short time. Can we assume that the cells of the grass when reaching the paunch are broken up to such an extent to liberate the lethal dose within 15 minutes? In view of the fact that the paunch is alkaline, thus not at the optimum pH for the ferment, in view of the fact also that only a small proportion of the cells are broken up within the

first hour, we may be justified in thinking that the glycoside does not liberate enough prussic acid and that therefore the latter is not the only toxic substance involved in killing the animal. The experiments both of Peters and of Petrie are open to this doubt.

The question of elimination from the animal body is all important in this connection. Prussic acid on account of its high diffusibility will readily reach the blood stream, but it will, by this same property, be eliminated very quickly. The balance between elimination and the supply from the ingested material will determine to a large degree the toxicity of the dose. If the ingested material is slow to break up in the paunch, the animal will stand more than the lethal dose determined on pure chemicals.

This question that possibly another substance could be involved in these deaths should be seriously taken into account in future investigations. One way of testing the question would be to determine the prussic acid content of the material before it is fed. After the death of the animal the grass found in the paunch should be retested, to find out how much prussic acid *had actually been liberated*. The contents of the paunch would best be introduced into 95 per cent. alcohol to prevent loss during handling and transport to the laboratory. An experiment *in vitro* made with saliva and extract of the paunch would also show to what extent these juices are favourable or unfavourable to the liberation of prussic acid.

The experiment of Pease (1897) may also be mentioned in this connection. Pease claims that deaths of cattle in India from Johnson grass were really cases of nitrate poisoning. He was able to detect 20 per cent. of potassium nitrate in stems of the grass and in feeding this salt to the animals was able to reproduce some of the symptoms.

That cyanide is not toxic under all circumstances is borne out by some experiments of Loeb (1910). It is a well-known fact that the eggs of the sea urchin are killed by a pure solution of NaCl. The toxic effect of Na can be counterbalanced as Loeb has shown by sodium cyanide, a very peculiar effect, which the author tries to explain by the inhibitory action of cyanide on the oxidation.

## V. CONDITIONS IN THE ANIMAL FAVOURING OR PREVENTING TOXICITY.

In all cases of poisoning, the state of the animal previous to its eating the toxic plant must be taken into account, in cases of prussic acid poisoning more than in any others.

Swanson (1921) has shown that marked alkalinity and marked acidity have an inhibiting influence on the production of prussic acid. Thus the paunch which is alkaline and the stomach which is acid will decrease to a considerable extent the production of HCN. I have tested HCl in conjunction with pepsine and have found that the grasses crushed in a mortar emit more HCN in ordinary water than with HCl and pepsine.

The question of hunger, overstrain, bad health, thirst, drinking water before or after eating the toxic grass, should be taken into account. The effect of the toxic grass will largely depend on what

the animal had been eating before. Indeed Peters, Slade and Avery (1903) have shown that considerable doses of HCN can be given to an animal without detriment, provided it is also given an adequate amount of glucose or milk sugar. As glucose is produced by the action of ptyalin on starch, starch food may act as an antidote and so may milk. These facts may account for the many erratic results one gets in experimenting with these toxic grasses. Starch, milk and molasses should therefore be subjected to further tests for their value as antidotes or preventives.

Steyn has shown that sulphur is an excellent preventive against prussic acid poisoning (Geilsiekte). For further discussion of the question see Steyn "Toxicology of plants in South Africa" (1934).

## VI. EXTERNAL CONDITIONS LEADING TO TOXICITY OF THE PLANT.

Brünnich (1903) in quoting from a lecture by W. C. Quinell, gives the following list of "conflicting statements and theories . . . on the circumstances and conditions under which sorghum is believed to become poisonous."

1. "If sorghum is eaten in an immature condition.
2. When sorghum grows rapidly after rainfall.
3. When the plant is stunted by failure of rain or by frost.
4. When sorghum is attacked by insects during an exceptionally dry season.
5. A poisonous mould or fungus is supposed to be the medium of poison.
6. In some parts of India, the plant is said to be poisonous until the rains (monsoons) are over.
7. The poisoning is attributed to the potassium nitrate which, under certain circumstances, is precipitated in the stems of the plants.
8. Physiologic changes of growth of the plant owing to climatic disturbances, such as want of rain, excess of humidity, damp cloudy weather, or prevalence of extremely variable and unnaturally high temperature."

Brünnich (1903) in his article, states that these points were submitted to an experimental examination. It would be indeed very interesting to submit them to such a test. We find, however, very little of it in Brünnich's paper.

In the above list the age of the plant and the soil conditions are not mentioned. Brünnich has dealt with them to some extent in a later part of his paper. We find further reference to these factors in the following summary by Willeman and West (1915) which states the case so clearly that I shall give it *in extenso*:—

"Maxwell states that sorghum is not fed with safety until after the seeds begin to develop: Brünnich that it should not be fed until the seeds are fully matured, Avery says that the amount of hydrocyanic acid is greater in stunted plants, while Alway and Trumbull

found that yellow stunted plants contained less of the acid than the green, vigorous plants in the same field. Maxwell believes that the amount of the glycoside is dependent on the character of the soil, soils rich in nitrogen producing plants richer in the glycoside. Brünnich, in experiments with sodium nitrate in Queensland, found that the fertilized plants contained slightly more hydrocyanic acid than those unfertilized and concluded that heavy nitrogenous soils and favourable climatic conditions increase the amount of the acid. His findings were corroborated by Alway and Trumbull. Brünnich also found that millet (*Panicum mileareum*) behaved similarly to sorghum. Schröder and Dammann in Uruguay, report an increase in prussic acid due to the use of sodium nitrate as a fertilizer. Balfour noticed that plants infected with *Aphis sorghi* contained more hydrocyanic acid than uninfected plants."

The above remarks have been quoted with the object of showing how much still remains to be done to reach a sound view on the conditions affecting the prussic acid content of the grass.

#### (a) CLIMATE AND SOIL.

In verifying some of the above statements, Willaman and West (1915) found that nitrogen added to a poor soil may slightly increase the amount of HCN but that a fertile soil with abundant nitrogen will not show any effect on fertilization.

Pinckney (1924) "has determined HCN in sorghum plants grown in a greenhouse using three Minnesota soils low in nitrogen content and adding sodium nitrate in different amounts. The size of the plants, their colour, and prussic acid content were affected by the amount of nitrate applied".

Although it is thus demonstrated that the soil has a marked effect on the prussic acid production, which is interesting from an academic point of view, from a practical point of view the increase is not such as to warrant any further investigations in that direction. As pointed out by Willaman and West (1915) climate is a far more important factor than soil in the production of detrimental quantities of prussic acid.

As a drastic illustration to this fact we may mention a case described by Dunstan and Henry (1902). These authors quote Bonamé who showed that the dark coloured beans of *Phaseolus lunatus* in Mauritius yield more prussic acid than the pale ones. In Burma the pale buff semi-cultivated beans contain only a trace of prussic acid. But that it is not all a question of colour and variety is borne out by Guignard's investigations, who had obtained prussic acid from white beans. He has also shown that the relation of colour to prussic acid yield in wild plants of Java is not so clearly marked as in Mauritius.

Dr. Steyn has informed me orally of a case of *Cynodon transvaalense*, where the plant, growing in moist conditions, yielded no prussic acid, whereas the plants growing on a dry ridge showed a strong reaction. A similar observation was made by Narasimha Acharya (1933) on *Sorghum vulgare*.

Climatic influence on prussic acid production is clearly demonstrated, but its effect can not as yet be foretold.

.(b) DIURNAL AND SEASONAL VARIATIONS.

Diurnal variations in the production of prussic acid have frequently been recorded. Ravenna (1907) has found in sorghum an increase from morning to afternoon. Willaman and West (1916) have shown that there is a maximum at midday for the same plant. Marais and Rimington (1934) working on *Dimorphotheca cuneata* Less. found an increase in prussic acid content from early morning to noon, which they think "suggests a correlation with intense photosynthetic activity."

Narasimha Acharya (1933) working on *Sorghum vulgare* found an increase of prussic acid production from early morning to about 2 p.m., after which there is a slight decline till 6 p.m. followed by a rapid decline at night. This last author also is of the opinion that there is a correlation with photosynthesis. The writer has noticed in *Eustachys paspaloides* that there is more prussic acid in the morning than in the afternoon.

Yap (1920) has shown on sugar cane in the Phillipines that the photosynthesis of the leaves is more active in the morning than in the afternoon. They were most active from 8 to 10 a.m. and then there was a decrease from 10 a.m. to 4 p.m. This decrease after 10 a.m. does not seem to fit in with the above observations, yet one may assume that the nitrogen metabolism may lag and reach its maximum after the maximum of photosynthesis.

Much stress has been laid on the age of the plant and various workers have found marked differences in the prussic acid production as the plant grows older. The stems and leaves have been examined separately and it was generally found that the stems contain less HCN than the leaves and the leaves contain the acid in various degrees according to their situation on the stem.

Petrie (1913) in a series of grasses in New South Wales has tested the prussic acid content throughout the year and his experiments show the seasonal variations very well, and these, of course, are coupled with the age of the plant. Petrie has submitted his grasses to three tests, basing them on the assumption that the plant may contain the glycoside as well as the ferment, or the glycoside alone, or the ferment alone. The tests were carried out as follows:—

- (a) Chloroform test (probably with Guignard paper although not stated).
- (b) Emulsion test, in case ferment is absent and glycoside present.
- (c) Amygdalin test, in case ferment is present and glycoside is absent.

The names are quoted *verbatim* from Petrie, “+ a” denotes that case “a” gives positive results, “-” denotes negative results throughout. Here are a few cases.

	Jan.	Apr.	Aug.	Nov.
<i>Andropogon halepensis</i> Sibth var.				
<i>mutica</i> Hack ... ..	+ a	+ a	+ a	+ a
<i>Chloris petraea</i> Sw. ... ..	+ a	—	+ a	+ a
<i>Cynodon dactylon</i> Pers. ... ..	+ c	+ c	—	—
<i>Cynodon incompletus</i> Nees. ... ..	+ a	+ a	+ a	+ a
<i>Eleusine aegyptiaca</i> Pers. ... ..	+ a	+ a	—	+ a
<i>Eleusine indica</i> Gaertn. ... ..	+ a	+ a	—	+ a
<i>Lazarus ovatus</i> Linn. ... ..	—	+ c	—	+ c
<i>Paspalum scrobiculatum</i> Linn. ...	—	—	+ c	—
<i>Penisetum longistylum</i> Hochst. ...	+ c	—	—	

Investigations of the kind made by Petrie, establishing the existence of either glycoside and ferment or ferment alone should be encouraged. The grass containing the ferment only may prove dangerous when an animal has been eating another plant containing glycoside alone. A case of that nature has been described by Finne-more (1931) where *Acacia Georgina* Bailey Fv.m containing the glycosidase released prussic acid from *Eremophila maculata* containing the glycoside. We may one day come across a case where the grass supplies the ferment and another plant the glycoside. The more we know of the existence of the ferment in the plant, the better.

#### (c) WILTING.

The health of the plant plays an important rôle in the production or disappearance of prussic acid. Wilting is a state of bad health and in this state grasses often yield relatively great quantities of prussic acid. Willaman and West (1915) have also shown that adequate water supply is usually accompanied by low, and inadequate, by high prussic acid content. The water relation of the plant, in other words, hydration and dehydration affect the amount of prussic acid produced.

Wilting is an abnormal condition of the plant accompanied by a lowered vitality. Permanent wilting is highly detrimental. The process starts by an excess of transpiration over water supply. The interstices between the cells give up their water first and the air in those interstices becomes less and less saturated. This is what Livingston and Brown call incipient drying. In heliophilous plants there may be an excess of 20-30 per cent. of water content in the cells of the leaves. In ombrophilous plants this excess is only 1-3 per cent. The releasing of the excess water produces a considerable shrinking of the cells and the leaves as a whole. The cells then lose their turgidity entirely and the shrinking protoplasm draws the cell walls inward. This drawing in of the cell walls reaches a certain limit after which the wall snaps off from the protoplasm and causes a mechanical injury to the latter.



Incipient drying is not a dangerous process and can easily be checked by introducing the plant into a moist atmosphere. Permanent wilting however cannot be immediately checked and reversed by bringing it into a moisture saturated atmosphere. The chemical changes which the dehydration has produced are too deep seated to be reversed at a short moment's notice.

The subtraction of water from the protoplasm is not a pure physical process. By the dilution and concentration of the cell contents, changes in ionisation take place which are gradually compensated by buffer action. Then certain substances may precipitate at high concentration and many a reaction will take place in the concentrated protoplasm which the less concentrated normal conditions would forbid.

Wilting is accompanied in the majority of cases by a closing of the stomata. This closing of the stomata together with the lowered vitality of the plant decreases photosynthesis to a considerable extent, there will be a lack of sugar and a lack also of oxygen, in other words, a decrease of respiratory energy. Mme. Brilliant (1924) has shown that when the water content of the leaf falls below 25 per cent. an abrupt decrease of photosynthesis is produced, lowering the process to about one-quarter of its original value.

All these facts must be borne in mind when we are trying to find a chemical relation between wilting and the enhancement of prussic acid which this state produces. We shall refer to this question again later on.

## VII. INTERNAL CONDITIONS OF THE PLANT LEADING TO TOXICITY.

The chemical and energetic processes within the cell are extremely involved and we have arrived only at a broad and summary view of the whole mechanism. Several theories have been propounded on the question of prussic acid and plant metabolism and the best we can do is to explain those theories and discuss their value in the light of most recent knowledge.

### (a) THEORY OF GORIS (1921).

According to Goris the rôle of the glycoside is to protect the plant against toxic effects of certain substances like prussic acid, benzaldehyde, etc. In linking these toxic substances with a sugar the toxic effect is eliminated.

But there is an extraordinary contradiction between the formation of a glycoside for protection purposes and the subsequent releasing of the toxic substances by ferments supplied by the plant itself. The plants do not only decompose the glycoside when they are wilting, thus in an abnormal state, but the diurnal variation of the glycoside content shows quite clearly that the sugar and the aglycone are drawn into circulation again.

The idea of protection in this case is rather far fetched. The question whether a plant will make a glycoside is not so much a question of utility but a question of chemistry and catalysis. The fact that the plants containing glycosides also possess ferments to split them seems to indicate that the glycosides are storage products, whether temporary or for longer periods does not matter. Willaman and West (1915) contend that the diurnal variation shows that the glycoside in grasses is not a storage product. But the definition of a storage product is not so much based on the time for which the product is kept, but rather on the fact that an excess has been set aside for the time being. The rapid reintroduction of a storage product into circulation does not do away with the fact that it has been kept out of circulation.

Robinson (1930) has discussed this question too and shown some of its fallacies.

### (b) THEORY OF GAUTIER.

Gautier in 1872 contended that free nitric acid under the influence of formaldehyde produces  $\text{HCN}$ ,  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . The prussic acid was then supposed to enter into long chains with formaldehyde from which Gautier derived his protein molecule.

Menaul (1920) has given this theory a test in the following way:—

“Six flasks each containing 400 c.c. of water saturated with carbon dioxide, 2 c.c. of 40 per cent. formaldehyde and 1 gr. of potassium nitrate were tested as follows:

1. Two flasks were made alkaline to phenolphthalein with sodium carbonate.
2. Two were made alkaline to methylorange but acid to phenolphthalein.
3. Two flasks were made acid to methylorange.

The flasks were stoppered and placed in sunlight for one month.

Results were as follows:—

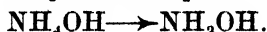
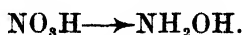
- No. 1: No  $\text{HCN}$ .
- No. 2: A trace of  $\text{HCN}$ .
- No. 3: 6 mg. of  $\text{HCN}$ .

“These results,” says Menaul, “when considered in connection with the fact that the sap of the plant is slightly acid and that the nitrate and formaldehyde are present indicate that prussic acid may be formed in plants by the action of formaldehyde on nitrates”.

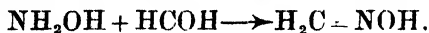
It is a pity that Menaul has not made a control in complete darkness and that he has not extended the experiment to other organic acids and other aldehydes. Latham in 1886 also attached a great importance to cyanogenetic radicals in the synthesis of animal proteins.

## (c) THEORY OF MEYER AND SCHULZE, 1884.

Meyer and Schulze supposed that nitric acid through reduction, and that ammonia by oxidation may lead to hydroxylamine.



Hydroxylamine in combining with aldehydes and ketones would form aldoximes.



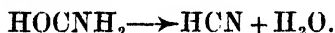
These aldoximes and ketoximes were finally supposed to lead to an amino-group.

The theory does not make any statement about prussic acid. We are referring to it here because Bach based his theory on these considerations.

## (d) THEORY OF BACH (1897).

Bach has carried the idea of Meyer and Schulze a step further. The nitrates are supposed to produce a certain amount of free nitric acid under the influence of organic acids. Nitric acid in the presence of formaldehyde would produce hydroxylamine. This, in agreement with Meyer and Schulze, would lead to formaldoxime.

The latter may then undergo transformation into the isomeric formamide. Formamide finally may undergo dehydration and yield prussic acid and water



This represents the dehydration theory of Bach. That this reaction can take place had already been shown by Scholl in 1891.

Thus supposing formamide is formed in the plant, dehydration by wilting would lead to the formation of prussic acid. The theories of Gautier, Meyer and Schulze, and Bach would also account for the increase of prussic acid through an excess of nitrates in the soil and in the plant.

## (e) THEORY OF TREUB (1907).

The theory of Treub is much more likely to be of some value because it is held in general terms and does not attempt to describe the details of the process. Gautier has already expressed the idea that prussic acid is an intermediate step to the proteins. He has spoilt his claim to priority in a way, by putting forward too precise an idea of how he thought this process could be brought about. As these supposed reactions were purely inventions based on scanty facts the otherwise excellent idea of HCN being a step towards the proteins, was spoilt.

Treub resting within the general idea of Gautier tried to show by experiments that there is much to be said in favour of it. His principal arguments, based on observations made on *Pangium edule*, *Phaseolus lunatus*, *Indigofera*, *Alocasia* are the following:—

1. The presence of free and bound prussic acid tends to show that it is involved in metabolism.

2. The amount of HCN increases with the activities of the leaf.
  3. In *Alocasia macrorrhiza* the production of HCN is limited to the green parts, which means that in those parts the nitrogen metabolism is highest.
  4. In old leaves the HCN production is reduced as the metabolism is reduced.
- Before the leaves are shed they are usually free of HCN.

Treub has also suggested a modification of Gautier's theory. According to Treub the production of prussic acid is not directly dependent on energy derived from light, but is influenced by the quantity of sugar present. The reduction of nitric acid would be brought about by the sugar.

#### (f) OBJECTIONS TO THE THEORY OF TREUB.

Rosenthaler (1922) has tested the theory of Treub by some experiments which were guided by the idea that if HCN is an intermediary product in plant metabolism it should be present in all plants. To prevent any source of error Rosenthaler has not used maceration for this experiment. He expelled prussic acid by a current of air after mincing the plant material. (The mincing may be a source of error.)

Out of 80 plants tested in such a way, 56 positively showed prussic acid. Rosenthaler rightly remarks that this fact in itself although favourable to the hypothesis of Treub, may not be considered as a definite proof because it does not show how prussic acid is produced and whether it is a product of synthesis or decomposition.

In order to obtain some more information on this point Rosenthaler injected an amino acid into sorghum. There is a definite stereochemical resemblance between phenylalanin and benzaldehyde-cyanhydrin, tyrosin and p-oxybenzaldehyde-cyanhydrin, valin and acetonecyanhydrin. It is also known that HCN can be obtained by the oxidation of amino acids.

Rosenthaler used tyrosin for his injection. If the idea of Treub is correct, he says, then the injection of tyrosin should induce a decrease in prussic acid. *Sorghum nigrum* was injected and showed a definite increase in HCN.

It may be recalled here that Ravenna and Zamorani (1910) have tested an injection of asparagin into Sorghum and found a decrease of HCN.

Rosenthaler's postulate that tyrosin should decrease the prussic acid content, because this amino acid resembles p-oxybenzaldehyde-cyanhydrin, rests on a very slender basis.

As a matter of fact we may *a priori* even expect the reverse, viz. than an excess of tyrosin will be transformed into p-oxybenzaldehyde-cyanhydrin and thereby increase the HCN content. But the processes involved when making a violent interference such as an injection, are so intricate that our conclusions are but wild guesses.

Rosenthaler's experiments are neither for or against Treub's hypothesis and admit of hardly any conclusion.

Oppenheimer (1925) and Stekelenburg (1931) are against Rosenthaler's conclusions.

Stekelenburg (1931) has made a series of experiments with a view to verifying the hypothesis of Treub. He has examined *Pangium edule*, *Phaseolus lunatus*, *Prunus padus* and *Prunus laurocerasus*. Stems, leaves, seeds and seedlings were submitted to a test. The method used for determination of HCN was that of Verschaffelt, with a temperature of 60° C. and maceration during 20-22 hours. The method seems open to criticism.

It would seem that Stekelenburg has drawn a series of rather sweeping conclusions from his experiments. We shall discuss their value partly here and partly under the heading of facts in favour of the theory of Treub.

Germination of *Phaseolus lunatus*: during germination the amount of HCN increases in the plant and then, as the cotyledons shrink, it decreases.

Stekelenburg concludes from this experiment that the cyanogenetic glycosides function as carbohydrate reserves. The releasing of HCN is not necessary because in his opinion the plant is drawing enough nitrogen from the soil. He points to the fact that Ravena has found the same phenomena of increase followed by a decrease on a soil devoid of nitrogen. According to Stekelenburg the experiment would prove that HCN is derived from some organic compound.

But the experiment does not warrant any such far-reaching conclusions and can be well interpreted in favour of Treub's hypothesis.

Buds and stems of *Prunus padus* and *Prunus laurocerasus*. The prussic acid content was measured before budding and then after, on cut twigs kept in the dark, cut twigs kept in light and on twigs still attached to the plant in light.

In *Prunus padus* the HCN of the stems remains practically constant. There is an increase of HCN in the buds under most of the above-mentioned conditions. In *Prunus laurocerasus* the etiolated buds (cut twig in the dark) showed a decrease, while the others manifested an increase.

The constancy of the HCN in the twigs and the increase in the buds tend to show that there is no migration of the acid. The facts do not in themselves support the idea which Stekelenburg here again emphasizes that cyanogenetic glycosids are storage products. In one case darkening had no effect and HCN increased, in the other it had an effect and decreased the prussic acid content.

#### Leaves.

During the day *Prunus laurocerasus* increases its HCN and maintains it constant during the night. There is no migration. The decrease starts in the dark, after the starch has disappeared. Leaves floating in 1 per cent. glucose sol. increase their HCN, whereas in pure water there is a decrease.

These results again are not in themselves of any support to the theory of reserves.

The more important fact which arises out of these experiments is that HCN increases when no nitrogen is offered to the plants. Yet the experiments are not of a conclusive character because the author has overlooked the possibility that the leaves may contain a considerable reserve of nitrates and they need very little to keep alive.

The influence of nitrates was tested in the following way. Leaves were floated on 0.1 per cent. nitrate solution and showed a decrease of HCN. When glucose was added an increase was noted equal to the increase above when the sugar was given alone. A 1 per cent. asparagin solution showed a decrease but in conjunction with sugar showed a marked increase. The author concludes that nitrates are not necessary for an increased HCN production. This conclusion meets with the same objection as above. If the plant contains enough nitrates in reserve, an excess will only be detrimental. The administering of sugar may have another effect. The effect of asparagine is still mysterious and no conclusion can be drawn from it.

Stekelenburg concludes from his experiment that HCN is not the first visible assimilation product of nitrogen, that therefore the hypothesis of Treub is erroneous. He further contends that HCN is a by-product derived from higher nitrogen compounds and has no importance in the N-metabolism. Transport of HCN does not take place. HCN may have a certain value as nitrogen reserve.

While we agree that the cyanogenetic glycosides are temporary storage products, we cannot subscribe to the author's conclusions with respect to the theory of Treub. The experiments in themselves, though a valuable contribution, do not carry that element of conviction, nor are they to the point. They simply do not permit of any definite conclusion with respect to the hypothesis of Treub and may, as we will show, just as well be used to confirm it.

#### (g) FACTS IN FAVOUR OF THE THEORY OF TREUB.

Greshoff, Ravenna, Dunstan and Henry, Oppenheimer and many others are in favour of the Theory of Treub.

To Greshoff's mind (1906) the wide distribution of prussic acid throughout the plant world, in a large number of families, is an indication of the importance of the acid in those cases where it is linked with acetone (acetone-cyanhydrin); it may possibly be an intermediate product in protein synthesis, in such plants as *Panguim edule*, *Linum usitatissimum*, and *Phaseolus lunatus*. He thinks, however, that in such cases where the acid is linked with benzaldehyde such importance may possibly not be attached to it. But it is difficult to see why in the one case prussic acid should be part of metabolism and why in the other it should not. The combination which the excess prussic acid will undergo, depends on whether benzaldehyde or acetone is present and these products are in themselves no indications of the purpose for which the acid is produced.

Ravenna (1912) also accepts the theory of Treub and thinks that HCN is a stage from nitrates over the amines to proeteins. He thought that this idea was strengthened by the fact that asparagin injected into the plant decreases the amount of HCN. But Czapek points out that aromatic substances produce the same decrease so that this argument is of as little value as the one forwarded by Rosenthaler for the opposite effects.

Ravenna (1912) also pointed out the fact of diurnal variation and thinks that prussic acid is produced by nitrates and carbohydrates in the presence of light. He also showed that the maximum prussic acid is produced in the leaves.

The diurnal variations in the prussic acid content of grasses is a striking fact which speaks in favour of Treub's theory. These rapid variations show that the excess acid is temporarily stored away and very rapidly also brought back into circulation. The theory is also supported by observations made in the shade and in the sun. Dr. D. G. Steyn has informed me orally that at 8 a.m. *Cynodon transvaalense* showed a high prussic acid content in the sun and a few yards away, in the shade showed none. Here evidently the higher activity in the sun will naturally produce an excess.

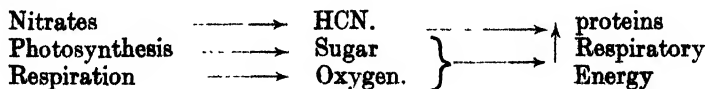
The increase of HCN by an abundant supply of nitrogen fertilizers also is in favour of Treub's theory.

We may also derive some arguments in favour of the theory from our consideration on wilting. By the lowered photosynthesis and the lowered supply of energy for the endothermic processes, the creation of new proteins will be very slow. Supposing this lowered energy supply does not affect the intermediate products as much as it does the end products, wilting would inevitably produce an excess of prussic acid if this substance is involved in the metabolic process., Henrici (1926) has considered this as a possible explanation too.

On the other hand we may, under normal circumstances, increase prussic acid if by an excess supply of nitrates we increase the rate of production of the intermediate products.

The process could be summarised as follows on the basis of Treub's idea.

#### 1. Normal Process.



#### 2. Wilting.

Decrease of respiratory energy; decrease of protein synthesis therefore excess prussic acid through accumulation of intermediary products.

#### 3. Normal energy supply but excessive supply of nitrates.

Result excess HCN.

#### 4. Excessive activity in the sun.

General increase of activity, increased transpiration, increased circulation. If the increase in circulation is higher than the increased protein synthesis, the case would correspond again to No. 2 or No. 3, accumulation of intermediary products.

Apart from the facts that Treub himself has pointed out, we may draw some arguments in his favour from the experiments of Stekelenburg (1931).

Seedlings and buds are known for their very high activity; thus naturally one would expect a high prussic acid content. It would be rather strange if those two would produce HCN and sugar for storage products. The facts seem to point rather in the other direction that an excess of sugar and HCN produced during high activity, meet and forcibly have to combine to form the temporary glycoside.

That darkening of the buds has no effect can be expected because these organs generally carry an excess of sugars and other nutrient substances, so that the lessening of photosynthesis will not affect them so much. In *Prunus laurocerasus* the darkening of the buds had a decreasing effect in Stekelenburg's experiments, so that all depends on the amount of reserves they contain.

The darkening effect on adult leaves is very marked, as can be expected, because they have very little reserves and are dependent on direct photosynthesis. As Stekelenburg points out the decrease in the dark starts when all the starch is used up, that means when the respiratory energy goes down. This is borne out by the experiment where leaves are floating on 1 per cent. glucose solution. The sugar here is taken in, not for the purpose of storing it, but for respiration and synthesis. An excess may yet be stored. But the abundance of sugar enhances respiration and thus the general activity of the plant.

That the nitrates do not produce an increase of HCN when offered to the leaves proves nothing. The leaves may contain an excess of nitrates already and offering them more will not help.

All experiments of Stekelenburg tend to show that active photosynthesis is coupled with high prussic acid content, confirming thus other experiments on the same factors.

Taking into account all facts, even those of Rosenthaler showing how widespread HCN production is in plants, we would feel inclined to grant the hypothesis of Treub, the title of a good working hypothesis.

This does not wholly do away with the possibility of creating prussic acid by other means, such as those described by the theory of Bach or shown by the experiments of Plummer (1904) who obtained prussic acid by oxidation of albumins. Aslander (1928) has shown that cyanides decompose rapidly in the soil and he ascribed the action to micro-organisms. Emerson [quoted from Czapek (1922)] has discovered that *Bac. pyocyaneus* digests proteins in an acid medium with the production of HCN.



**VIII. EFFECT OF FREE PRUSSIC ACID ON THE PLANT.**

The detrimental effect of prussic acid on the respiration of an animal is well known. It is ever so much more amazing that the abundance of that acid in plants does not seem to be injurious to plant cells.

In conjunction with the question of respiration and the effect of HCN, we should briefly recall the two theories which have a bearing on the question.

The theory of Warburg centres around the activation of oxygen which to his mind is done with the help of iron. The iron, according to this theory, in passing from a lower to a higher valency would be capable of producing peroxides of ever higher oxidising power. In this theory the inactivation of respiration by HCN would be explained in assuming an inactivation of the iron (ferri-form) by the prussic acid. If it were so, the plant does not seem to suffer much from such inactivation, whereas the animal is killed very rapidly.

According to the idea of Wieland, oxygen does not need to be activated. His theory centres around the activation of hydrogen brought about by dehydrogenation. Although for the chemist and with respect to the end products, direct oxidation is equivalent to dehydrogenation, yet for the organism they are not the same because different means are needed to bring them about. However excellent the idea of dehydrogenation may be, Wieland is at a loss to explain the inactivation of respiration by HCN. He tried to escape the difficulty by saying that prussic acid attacks the catalase and that the organism thus suffers from an excess of peroxides. The argument, however, is very weak.

In considering the considerable quantities of prussic acid produced, one wonders why the respiration of the plant is not impaired. The sorghums never seem to be free of the acid; if it is a part of the ordinary metabolism the plant organism can never be devoid of it. Some plants emit the acid freely, they live in an atmosphere constantly containing prussic acid, like *Nerium oleander*, and yet do not seem to suffer.

Yet a certain excess may still be harmful. Brinley (1927) has tested the effect of HCN on living cells. The acid seems to enter the cell as a molecule and not as an ion, although in water it dissociates to a slight degree. The rate of recovery of *Elodea* cells after having been placed in a dilute solution of HCN is a linear relation. The toxicity of HCN to the root hairs of *Limnobium* results in a uniform curve, suggesting a unimolecular reaction. HCN seems to increase the permeability of the cell membrane. (Quoted from Biol. Abstracts 1930, No. 7279.)

Hassebrauk (1928) has tested the effect of HCN on the maturity of seeds, among other plants also *Dactylis glomerata* and *Anthoxanthum odoratum*. The seeds were gassed with HCN and the effect proved favourable to after ripening and germination (quoted from Biol. Abstracts 1929, No. 17940)

Boresch (1929) undertook experiments to test whether the presence of HCN was related to the dormancy of the buds and to the breaking of their rest periods. No broad relationship was found, but "yes" would answer the question better than "no". (Quoted from Biol. Abstracts 1932, No. 6590.)

Cotte (1914) has shown how different plants vary in their sensitivity to HCN. This author tested *Triticum*, *Tropaeolum minor* and *Ricinus communis*. For the experiment the plants were kept in an airtight compartment of 0.64 cubic meters. These are his results:—

8 gr. HCN acting during 1 hour:	<i>Triticum</i> not affected. <i>Ricinus</i> not affected. <i>Tropaeolum</i> not affected.
10 gr. HCN acting during 1 hour:	<i>Triticum</i> slightly affected but surviving. <i>Ricinus</i> slightly affected but surviving. <i>Tropaeolum</i> no effect.
15 gr. HCN acting during 1 hour:	<i>Triticum</i> strongly affected, some dead after 27 days. <i>Ricinus</i> strongly affected, some plants killed. <i>Tropaeolum</i> slightly affected.
25 gr. HCN acting during 1 hour:	<i>Triticum</i> completely destroyed. <i>Ricinus</i> completely destroyed. <i>Tropaeolum</i> slightly affected but recovered.
25 gr. HCN acting during 2 hours:	<i>Triticum</i> completely destroyed. <i>Ricinus</i> completely destroyed. <i>Tropaeolum</i> injured but survives and flowers.

*Tropaeolum* shows thus a very high resistance towards the effect of prussic acid. *Triticum* and *Ricinus* are less resistant but the doses they can stand are still amazing.

No correlation could be established between anthocyan and prussic acid. It seems, however, according to my own experiments, that those parts of the leaves containing anthocyan produce more prussic acid than the green parts of the same leaf. The remarks of Henrici on this point are not quite clear.

## IX. PRUSSIC ACID IN GLYCOSIDIC OR NON GLYCOSIDIC FORM.

Willaman (1917) thinks that prussic acid exists in a glycosidic and a non-glycosidic form. Dowell (1919) contends that his experiments do not show the presence of the non-glycosidic HCN. But Willaman may be right with the restriction that the non-glycosidic form cannot last very long and if there is enough sugar present the glycoside will be immediately created.

# HYDROCYANIC ACID IN GRASSES.

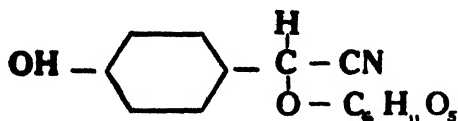
Narasimha Acharya (1933) thinks that there are at least three forms in which prussic acid is present:—

1. "Free prussic acid" formed by enzymic hydrolysis of "labile" prussic acid, destroyed by 10 per cent. sulphuric acid and steaming.
2. "Labile prussic acid" liberated by simple steaming. Destroyed by 10 per cent. sulphuric acid.
3. "Bound prussic acid", liberated by enzymic action, destroyed by heating and 10 per cent. sulphuric acid.

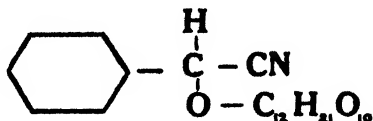
This is an interesting point which would deserve further investigation especially in view of throwing some light on the theories of prussic acid production as an intermediary stage of plant metabolism.

The glycosides so far isolated from grasses are amygdalin and dhurrin. (Dunstan and Henry 1902.)

The chemical composition of dhurrin is as follows:—



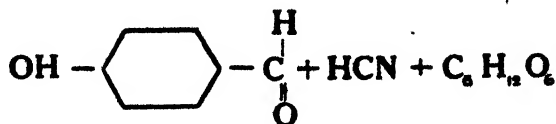
It will be useful to compare it with the well known amygdalin.



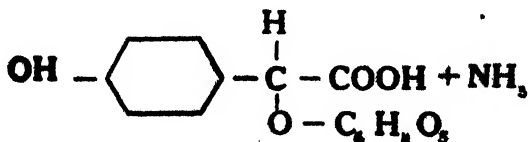
There is an extremely close resemblance between the two substances. The main difference lies in the sugars, dhurrin being coupled with a monosaccharid while amygdalin is linked up with a disaccharid. Moreover, dhurrin possesses a hydroxyl in para-position.

The effects of acids, emulsin and alkalis on dhurrin are the following:—

1. Hydrolysis by acids and emulsin:



2. Hydrolysis by alkalis:



producing dhurrinic acid and ammonia.

Considering the first fact, one would assume that HCl must forcibly increase the liberation of prussic acid. But as Swanson (1921) and the writer have found, the reverse is the case, HCl has an inhibiting influence when acting on the plant. This may be due to an effect on the ferment; the case needs some closer investigation. The influence of alkalis on the plant tends also to diminish the HCN. This may also be due to a direct effect on the ferment which works at pH 4.6, or to the above hydrolysis by alkalis.

Thus these two reactions need some more careful investigations, when considering the effect of the alkalinity in the paunch and the acidity in the stomach.

Judging from the link between the nitrile  $-CN$  and the rest of the molecule, one would *a priori* admit that the ferment which is capable of splitting off HCN from amygdalin will be capable of doing so also from dhurrin. This has been amply verified. Yet it should not be overlooked that the presence of the hydroxyl in dhurrin may, under certain circumstances, render the action of emulsin difficult, if not impossible.

The ferment that is capable of splitting amygdalin into its components is the well-known emulsin. It should be recalled at this juncture that emulsin is by no means a pure ferment and is composed of a series of components which are difficult to isolate. Its first component is an amygdalase which splits the disaccharide (called amygdalose) into glucose and d-benzaldehyde-cyanhydrin- $\beta$ -glucoside; the latter substance is prunasin. The first component of emulsin will not come into action for dhurrin, because as stated it only possesses a monosaccharid and could be called an oxy-prunasin.

The second component of emulsin, a prunase, splits off glucose from prunasin and will probably do the same for oxy-prunasin with the restriction mentioned above.

The third phase of fermentation is supposed to be performed by an oxynitrilase which would split off HCN from the cyanhydrin. The question how this oxynitrilase acts and whether it is a real ferment or not is not as yet settled, the reader will find a detailed discussion of the question in Oppenheimer: "Die Fermente."

The nature of the ferment present in the grasses which is capable of splitting dhurrin, has not received enough attention. Is this dhurrase in any way similar to emulsin in that it is a mixture of prunase, oxynitrilase and other ferments? If it were only an oxynitrilase its ferment nature may be doubted on the same grounds as that of the same component of emulsin. Attempts should be made to isolate this ferment and investigate it in all fermentative activities. There is no doubt of the existence of this ferment. In most cases, to obtain HCN, the leaves need just to be crushed or treated with chloroform vapour, so that ferment and glycoside may diffuse and react. Dunstan and Henry (1902) say that provisionally the ferment of *Sorghum vulgare* can be considered identical with emulsin.

**X. PRUSSIC ACID AS AN ORGANIC COMPOUND.**

Prussic acid is a tautomeric substance which occurs in two forms.



When replacing the hydrogen in these two isomers by organic radicals we obtain from the first, the nitriles, and from the second the isonitriles. The latter seem to be much more toxic than the former.

The salts of the acid are generally a mixture of both isomers and are difficult to separate.

By hydrolysis the nitriles lead to an organic acid,  $\text{R}-\text{COOH}$  and the isonitriles to an amine  $\text{R}-\text{NH}_2$ . A mixture of these two derivatives of prussic acid has thus already an amphoteric character.

We may possibly have a clue here to the second toxic substance which accompanies prussic acid, to which we have alluded, in the beginning. If prussic acid and its derivatives are in some way linked up with the protein metabolism it is very likely that both nitriles and isonitriles will be produced. They have both been found in plants. One of them only seems to form the glycoside, viz. the nitrile. The isonitriles may thus form a series of toxic substances which are not all detected by our prussic acid tests. This point, therefore, deserves serious investigation.

Some of the properties of prussic acid may interest us here with respect to precautions to be taken during extraction.

An aqueous solution of  $\text{HCN}$  is unstable and leads to ammonium formate. The pure acid is rapidly decomposed by concentrated  $\text{HCl}$ , with production of formic acid and ammonium chloride.

The first fact should be borne in mind when keeping a solution of  $\text{HCN}$  after extraction. The second is important with respect to the influence of  $\text{HCl}$  on the production of prussic acid by the plant. Although the concentration of  $\text{HCl}$  used in our investigations is very low, yet the decomposition of  $\text{HCN}$  may not be negligible. The influence of hydrochloric acid on the prussic acid production seems to be very complex. Although it is capable of hydrolysing dhuririn, its probable influence on the ferment and its direct effects on prussic acid itself, decrease the production of  $\text{HCN}$  to a very large extent.

The salts of prussic acid undergo decomposition when boiled in an aqueous solution. They produce a formate and ammonia. This fact should also be taken into consideration in all quantitative extractions.

**XI. EXPERIMENTS.**

All investigations described below have been made with the help of Guignard paper. This paper is prepared as follows:—

5 grams of sodium carbonate and 0.5 gr. of picric acid are dissolved in 100 c.c. of water. Strips of filter paper are dipped in this solution and then air dried. When the strips are still damp they are introduced into a well stoppered test tube. The test should always be made with a slightly damp paper.

The above test is a qualitative reaction and gives no information as to the origin and actual quantity of the HCN liberated. It may, however, be considered, to a certain extent, as a quantitative test when we observe the degree of darkening of the paper and the time it takes to reach a certain shade.

(a) EXPERIMENTS ON *Sorghum verticilliflorum*.

1. Leaves not crushed + chloroform—very rapid reaction, the Guignard paper turns violet within 7 minutes.
2. Leaves not crushed without chloroform—no sign of prussic acid even after 24 hours.

These two experiments distinctly point to a fermentative process and show that in this case, at that particular instant, all prussic acid was glycosidic.

3. Crushed leaves, without chloroform—paper turned more brown than violet, but darkens just as much as No. 1, though slower.

The crushing brings the glycoside and the ferment together.

4. Crushed leaves + HCl (0.1N HCl added just to cover the crushed leaves)—positive reaction but weaker than No. 3.

At 39° C there is an increase in the production of HCN. Probably this temperature drives out a certain amount which was dissolved.

This confirms the findings of other authors on the inhibitory effect of HCl on the prussic acid production.

5. Uncrushed leaves + HCl—(0.1N HCl added just to cover the leaves)—no sign of prussic acid after 24 hours.

Heating to 39° does not help.

This is rather strange in view of the fact that dhurrin can be hydrolysed by HCl.

6. Base of stem (containing much anthocyan) + chloroform—strong positive reaction within 7 minutes.
7. Base of stem crushed, without chloroform—slight sign of prussic acid after 4 hours; stronger after 24 hours, but not as strong as No. 6.

The crushing in this case does not seem to bring the glycoside and the ferment so intimately into contact as chloroform does by affecting the permeability. The crushing may bring about a series of reactions, which hamper the fermentation of the glycoside.

8. Old stems + chloroform—positive but very weak even after 24 hours.
9. Runners + chloroform—positive but very weak even after 24 hours.
10. Runners crushed, without chloroform—positive but very weak even after 24 hours.

11. Leaves that have been lying on the table for  $1\frac{1}{2}$  hours tested again with chloroform—very strong reaction.
12. Hay.—Six open tubes containing fresh grass with Guignard paper (no chloroform) were introduced into a desiccator containing  $H_2SO_4$  conc. This is a rapid hay production. The grass was tested after 2 days. The Guignard paper was only slightly tinted by escaping prussic acid. This shows that the process of drying does not in all cases result in an emission of prussic acid, although by transformation of HCN into other substances it may result in a loss.

The tubes were tested by heating to  $52^\circ C$  with the following liquids (leaves partly immersed):—

- (a) 1 tube dry grass + dilute HCl—strong positive.
- (b) 1 tube dry grass + dilute ammonia—strong positive.
- (c) 1 tube dry grass + distilled water—strong positive.

The hay thus contains a considerable amount of prussic acid, the liberation of which under these particular conditions does not seem to be hampered by acidity or alkalinity.

Hay from the same plant was retested 5 months later, but no longer showed signs of prussic acid.

#### (b) EXPERIMENTS ON *Eustachys paspaloides*.

Four tins each containing a plant of *Eustachys paspaloides* were brought to my laboratory. The leaves were immediately tested and showed strong production of prussic acid. Four days later there was no longer any sign of prussic acid. As the plants had been transplanted they were very weak and probably used up their prussic acid in urgent metabolism.

The following tests were made on the grass:—

1. Spikelet.
  - (a) With chloroform—no sign of prussic acid.
  - (b) Without chloroform—no sign of prussic acid.
2. Leaves.
  - (a) With chloroform—strong production of prussic acid.
  - (b) Without chloroform ( $21^\circ C$ )—no sign of prussic acid.

Here again there seems to be no non-glycosidic prussic acid.

The experiments with higher temperatures were made in the following way: The grass blades were cut to the length of 6 cm. and introduced into an 8 cm. test tube. At the bottom of the tube 0.5 cm. of water were placed and a Guignard paper was suspended inside by the help of the stopper. This test tube was then immersed in a water bath of the required temperature.

- (c) Without chloroform  $45^\circ C$ .—no sign of prussic acid after 2 hours. The tubes were left overnight at ordinary temperature. In the morning after 18 hours the paper was dark brown from the liberation of prussic acid.

- (d) Without chloroform at  $70^{\circ}$  C., Guignard paper turns brown after 5 minutes. This high temperature seems to have much the same effect as chloroform on permeability. If the plants, having remained at  $70^{\circ}$  C. for some minutes, are tested with chloroform at ordinary temperature, there is no production of prussic acid. Most probably the ferment has been killed at  $70^{\circ}$  C.
- (e) Without chloroform  $59^{\circ}$  C. This is probably the temperature of wilting grass on a hot day. The grass kept at  $59^{\circ}$  C. for 10 minutes shows a strong production of prussic acid. If the same grass is again tested at ordinary temperature with chloroform, there is no sign of prussic acid. But after 16 hours there is a slight recovery. The ferment still seems to be injured but not to the same extent as at  $70^{\circ}$  C.

#### *Stems.*

Tested with chloroform—no sign of prussic acid even after 48 hours.

A normal solution of HCl does not produce any prussic acid on uncrushed leaves after 2 hours at ordinary temperature.

If old plants devoid of HCN are cut down and left to grow, the young leaves show HCN again.

The parts of the leaves containing anthocyan show a stronger production of prussic acid than the purely green parts of the same leaves.

Leaves collected in the morning produce more HCN than those collected in the afternoon. This confirms the observations of many other investigators.

*Hay Production.*—Four tubes with fresh leaves were kept in a desiccator over sulphuric acid. A strip of Guignard paper was introduced into each tube. There was no sign of prussic acid during drying. The tubes were kept 4 days under these conditions. After that the following tests were made:—

1. The hay was moistened and heated to  $70^{\circ}$  C. in a water bath. Strong reaction of prussic acid.
2. The hay plus 1 per cent. HCl acid covering 1 cm. of the base of the 6 cm. leaves left at ordinary temperature. A slight but very distinct amount of prussic acid is produced within 24 hours.
3. The hay plus 1 per cent. HCl heated to  $70^{\circ}$  C. produces a slight amount of prussic acid.

Here we have the inhibiting effect of HCl again in a very marked degree.

4. Hay alone heated dry to  $70^{\circ}$  C. No sign of prussic acid. After addition of a few drops of water at that temperature the Guignard paper immediately showed the reaction.



The leaves of *Eustachys paspaloides* were squashed in a mortar and pounded with sand in order to break them up. They were then treated with standard acetate pH 4.6 which is the optimum pH for emulsin.

Emulsin was extracted from almonds.

1. The crushed leaves + standard acetate in a test tube showed no signs of prussic acid.
2. The crushed leaves + standard acetate + emulsin showed strong signs of prussic acid.

The experiment with emulsion is often deceptive because the ferment is a paste which does not allow the gas to escape so easily.

Crushed leaves (without standard acetate) were tested with HCl and pepsin. This was made to reproduce the conditions in the animal's stomach.

1. Crushed leaves without chloroform—showed signs of prussic acid after 10 minutes.
2. Crushed leaves with chloroform—strong production of prussic acid.
3. Crushed leaves + 1/1000 HCl (acid just covering the leaves)—no sign of HCN at 18° C., but distinct sign at 36° C.
4. Crushed leaves plus 1/1000 HCl plus pepsin—weak sign at 18°, distinct increase at 36° C.

As compared with the production of HCN under chloroform, the production of acid under pepsin and HCl is negligible. This is another proof of the inhibitory effect of HCl.

An illustration of the variation in the prussic acid content on two consecutive days is given by the following experiment. Five plants were examined and showed the following reactions:—

- No. 1.—No sign of HCN.
- No. 2.—No sign of HCN.
- No. 3.—Strong production of HCN.
- No. 4.—Weak production of HCN.
- No. 5.—No sign of HCN.

Twenty-four hours later the situation was as follows:—

- Nos. 1, 2, 3 and 4.—Weak production of prussic acid.
- No. 5.—Strong production of prussic acid.

Some of my observations tend to show that a cold wind will considerably reduce the production of the acid.

### (c) EXTRACTIONS.

The following experiments may serve as an illustration of the difficulties and pitfalls in the process of extraction. They may be useful in future investigations to avoid sources of error.

*Experiments on Sorghum verticilliflorum.*

Plants tested on their arrival with chloroform—positive, strong.

1. Alcohol extract 95 per cent. plus a small amount of calcium carbonate.

Tested the extract by suspending a Guignard paper above it in a test tube—

- (a) cold—negative;
- (b) warm—negative.

Thus the 95 per cent. alcohol extract, although it contains both ferment and glycoside, will not allow of any liberation of HCN.

2. Evaporated alcohol on water bath (70° C.) and residue taken up with water.

Test of the solution:—

Cold.—Negative.

Warm, 61° C.—Positive.

The method so far is safe and can be employed without fearing any loss of the acid by evaporation.

3. Alcohol extract 42.5 per cent. plus calcium carbonate.

This extract was tested:—

- (a) During extraction—strong production of HCN.
- (b) Cold after extraction—strong production of HCN.
- (c) Warmed again after 12 hours, 60° C.—strong production of HCN.

Thus alcohol of a lower concentration is not at all safe for extraction purposes, because the losses during the process are far too high.

To obtain some information about the influences of metals on the production of HCN the following experiment was made:—

100 c.c. of extract No. 3 was treated with 50 c.c. 50 per cent. ammonium oxalate.

The ammonium oxalate precipitates Fe, Mg and Ca. The treated solution was filtered and the filtrate examined. At 60° C. the Guignard test proves positive.

The subsequent addition of  $\text{FeSO}_4$ ,  $\text{MgSO}_4$  and  $\text{CaCl}_2$  makes no difference.  $\text{MnSO}_4$  and  $\text{AlCl}_3$ , however, have a distinct inhibitory effect.

Purification with lead acetate can be done in two ways. It can either be added to the alcohol extract No. 1 or to the extract No. 2.

In the first case when lead acetate is added to the alcohol (95 per cent.) extract, there is a positive Guignard reaction before filtering. After filtering the filtrate shows but a weak sign of prussic acid. When filtrate and residue on the filter are brought together again, there is no reaction on Guignard paper.

It may be concluded from this experiment that lead acetate brings down the ferment, which being concentrated at the bottom of the flask reacts strongly on the glycoside for a short while. But the precipitation being accompanied by denaturation of the ferment, the fermentive action soon stops.

This seems to be borne out by the second experiment. When lead acetate is added to extract No. 2 (water) and lead is eliminated by ammonium oxalate, the following results are obtained:—

- (1) At room temperature—
  - (a) filtrate—negative;
  - (b) filtrate + emulsin—positive.
- (2) At 65° C.—
  - (a) filtrate—Guignard positive but weak;
  - (b) emulsin alone—negative.
  - (c) filtrate + emulsin—Guignard positive strong.

This shows quite clearly that lead acetate precipitates the ferment. If the ferment is subsequently replaced by emulsin the positive results are obtained again.

The experiments also demonstrate that both the ferment and the glycoside are soluble in alcohol, but that most probably the alcohol itself or another substance equally soluble in alcohol prevents them from reacting. This may serve as a basis for finding an antidote for poisoned animals. The substances which are dissolved in 95 per cent. alcohol are fats, essential oils, phytosterines, phosphatides, fatty acids, glycosides, resins, tannins, chlorophyll, etc. Some of these substances should be tested by veterinarians to see whether they are of any use under the conditions of the digestive system of the animal to prevent further liberation of prussic acid. In all these experiments on animals there should be constantly borne in mind the statement made at the beginning of this paper, that prussic acid may not be the only toxic principle. We should also not forget that in the paunch there is a strong bacterial action which may increase the production of HCN.

The glycoside is not very soluble in ether. This was shown by the following experiment. Liquor No. 2 was shaken out with ether and the two liquids separated. The ether was left to evaporate and the residue taken up with water. This solution treated with emulsin gave a faint reaction. The liquor which had been separated from the ether was freed from the latter and was tested with emulsin. The reaction proved very strong.

I have only tried one adsorbent in an endeavour to separate the glycoside from the ferment. Polvaluminium hydroxide was used on the alcohol extract. The solution was filtered and the filtrate evaporated and then taken up with water. It proved positive to Guignard paper showing that neither glycoside nor ferment was extracted to any noticeable extent. Moreover, the polyaluminium hydroxide when added to this last solution had an inhibiting effect.

An acid water extract was also made with tap water + HCl to make it 0.1 normal. The extract alone heated to 65° C. shows strong production of prussic acid. When this solution is treated with lead acetate it becomes strongly fluorescent. The filtrate and the precipitate both show the fluorescence.

The precipitate was separated from the filtrate by filtration and the residue on filter tested with emulsin which proved positive. The filtrate was also tested with emulsin and proved positive. This is good evidence that the glycosid partly goes down with the lead precipitate and partly remains in solution. This fact should be borne in mind when lead acetate is used to clear an extract. It shows that the method of Dunstan and Henry can not be used for reliable quantitative determinations of the glycoside.

### SUMMARY.

The paper attempts to give a synoptic and constructive account of our present day knowledge on the problem of prussic acid in grasses and urges that the investigations should be made in a more academic spirit.

A list of 88 grasses is compiled indicating the authors who have dealt with them and other points of interest.

The methods of extraction are discussed to some extent and the errors which may occur in quantitative determinations are pointed out.

In discussing the lethal dose the view is expressed that possibly another toxic substance besides prussic acid may be involved in the rapid death of animals. A hint is given how to verify that contention, the important point being to find out how much HCN is *liberated* in the animal and not how much is *introduced*.

The question of antidotes is only briefly referred to.

The discussion of the external conditions leading the plant to toxicity shows clearly how climate and soil interfere with the metabolism of the plant. Climate and soil, diurnal and seasonal variations and the effect of wilting are discussed.

The origin of prussic acid in the plant is still an unsolved problem. The most important views and theories of the past and present are reviewed and on that basis, the theory of Treub is given the benefit of a good working hypothesis.

Numerous experiments show the various effects of HCN on the plant and demonstrate what strong doses it is capable of standing.

A discussion is devoted to the fermentation of dhurrin.

In the chapter "prussic acid as an organic compound" the view is expressed that the second toxic substance referred to in the beginning may possibly be an isonitrile which is much more toxic than the nitrile form producing glycosids.

The author describes some of his own experiments on *Eustachys paspaloides* and *Sorghum verticilliflorum*.

HCl has an inhibiting effect on prussic acid production, so has marked alkalinity and also pepsin + HCl.

During hay production no prussic acid escapes although some may be transformed into other substances. The hay still contains a considerable amount of prussic acid.

Heating the grass to 59° C. (probable temperature of wilting) and 70° C. releases as much HCN as the chloroform test.

Extractions made with 95 per cent. alcohol are safe, no prussic acid escapes during the process. 42.5 per cent. alcohol is not safe, the acid escapes during extraction.

Elimination or adjunction of Fe, Mg and Ca makes no difference, but Al and Mn have an inhibitory effect.

Lead acetate precipitates the ferment with denaturation. Lead acetate partly also precipitates the glycoside; a fact to be taken into account in quantitative tests.

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## Section VI.

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# Toxicological Studies.

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STEYN, D. G. . . . The detection of strychnine in carcasses and  
corpses.



## **The Detection of Strychnine in Carcasses and Corpses.**

By DOUW G. STEYN, B.Sc., Dr.Med.Vet., D.V.Sc., Veterinary  
Research Officer, Onderstepoort.

- I. Introduction.
- II. Tests employed in the detection of strychnine.
  - (A) Taste.
  - (B) Chemical tests.
    - (a) Precipitation tests.
    - (b) Colour reactions.
    - (c) Physical and chemical properties.
  - (C) Detection of strychnine by the dialysis method.
  - (D) Biological tests.
    - (a) The solvent.
    - (b) The animal.
  - (E) Quantitative estimation of strychnine.
- III. For what period after death is strychnine detectable in corpses and carcasses.
- IV. Discussion and recommendations as to the most reliable method of diagnosing strychnine poisoning.
  - (A) Methods of extracting strychnine.
  - (B) Detection of strychnine *intra vitam* and in corpses and carcasses.
  - (C) The most suitable tests for the detection of strychnine.
  - (D) Substances resembling strychnine.
    - (a) Chemical substances other than ptomaines.
    - (b) Ptomaines.
- V. Summary.

### **I. INTRODUCTION.**

IN forensic medicine it is of the utmost importance to know whether or not strychnine is present in corpses and carcasses in cases of suspected strychnine poisoning. The vital importance of this point is easily realised in cases of suspected malicious poisoning in human beings.

This investigation, the preliminary results of which are recorded in this article, was prompted by the fact that a difference of opinion exists among those concerned with forensic medicine as to whether or not, the biological test is essential in the detection of strychnine in corpses and carcasses. Some maintain that the taste and chemical (colour) tests yield sufficient evidence as to the presence of strychnine in extracts prepared from corpses and carcasses, whilst others are of opinion that it is essential that the results of the chemical examination be confirmed by biological tests.

## II. TESTS EMPLOYED IN THE DETECTION OF STRYCHNINE.

### A. TASTE.

Strychnine has an intensely bitter taste, which is still detectable in very dilute solutions. In the literature there is a striking discrepancy in the dilutions of strychnine in which this bitter taste is detectable: (a) Cloetta (1866) states that if 1 c.c. of a 1:250,000 solution of strychnine in distilled water be placed on the tongue it still has a bitter taste. On further dilution the bitter taste disappears. Some investigators state that a solution of 1:600,000 is still bitter, Cloetta however disagrees; (b) according to Gadamer (1924) the bitter taste of strychnine is still detectable in dilutions of 1:40,000-67,000; (c) Autenrieth (1928) and van Itallie and Bylsma (1928) state that the bitter taste of strychnine is still detectable in aqueous solutions of 1:670,000; (d) Glaister (1931) referring to strychnine writes "1 grain will impart to a gallon of water (1 in 70,000) a perceptible bitterness", and (e) according to Klein (1933) a dilution of 1:700,000 still has a bitter taste.

The above discrepancies are due probably, firstly to a difference in the susceptibility of the taste nerves of the different individuals, and secondly, to the fact that the different individuals took different quantities of the solutions to be tasted into their mouths. It is obvious that a bitter taste may not be perceptible when a drop of a very dilute solution of strychnine is placed on the tongue, whilst when 1 or 2 c.c. of the same solution is taken it will be bitter. When expressing a view as to the perceptibility of a bitter taste of strychnine in certain dilutions both the dilution and the quantity of the solution tasted should be mentioned. It is obvious that different dilutions of strychnine should not be tasted immediately after each other as the taste-nerves become exhausted very soon and hence are unable to record the bitter taste of a solution even if it is more bitter than the one tasted previously. An hour or more should be allowed to elapse between the tests.

The author was unable to detect a bitter taste in dilutions of strychnine beyond 1 in 200,000 in distilled water. A standard quantity of 1 c.c. of each dilution was placed on the tongue. The author failed to detect a bitter taste when only a drop of 1:200,000 strychnine was placed on the tongue.

## B. CHEMICAL TESTS.

Only *well purified extracts* should be used for chemical reactions (precipitation and colour tests) as impurities may interfere with the reactions.

(a) *Precipitation Tests.*

It was decided to ascertain the sensitivity of certain commonly used alkaloidal reagents for strychnine. The dilutions were prepared by dissolving strychnine sulphate in distilled water slightly acidified with sulphuric acid. The precipitating agents for alkaloids mentioned in the table given below were prepared according to prescriptions given by Fulton (1932):—

TABLE I.  
*Sensitivity of alkaloidal reagents for strychnine.*

Precipitating Agents.	Dilutions.							
	1: 1,000.	1: 2,000.	1: 4,000.	1: 8,000.	1: 16,000.	1: 20,000.	1: 30,000.	1: 50,000.
"Platinum Chloride"....	+	+	+	+	+	+	+	+
Potassium chromate.....	+	+	+	+	+	+	+	+
Potassium cyanide.....	+	+	+	+	+	+	+	+
Phosphotungstic acid.....	+	+	+	+	+	+	+	+
Phosphomolybdic acid.....	+	+	+	+	+	+	+	+
Wagner's reagent No. 1...	+	+	+	+	+	+	+	+
"Gold Chloride".....	+	+	+	+	+	+	+	+
Mayer's reagent.....	+	+	+	+	+	+	+	+
Mercuric sodium nitrite..	+	+	+	+	+	+	+	+
Picric acid.....	+	+	+	+	+	+	+	+
Tannic acid.....	+	+	+	+	+	+	+	+

+ + + = Very strongly positive (heavy precipitate).  
 + + = Strongly positive.  
 + = Positive.  
 ± = Faintly positive.  
 — = Negative (no precipitate).

The above tests were made by placing one drop of the different dilutions of strychnine sulphate on a watch-glass and then adding a drop of the precipitating agent. From the above table it is evident that Wagner's reagent No. 1 still gives a macroscopic recognisable precipitate with strychnine in dilutions of 1 in 20,000. The approximate amount of strychnine sulphate in a drop of a dilution of 1 in 20,000 is 0.0031 mgm. In the course of this article (see biological tests) it will be seen that the author adopted the following method of testing the extracts prepared from carcasses: The purified residue of the chloroform extract of the organs is dissolved in 1.5 c.c. of physiological saline, slightly acidulated with sulphuric acid. If one drop of this dissolved extract were macroscopically to yield a positive precipitation test with Wagner's reagent No. 1 there should be at least 0.074 mgm. strychnine sulphate contained in the 1.5 c.c. That is, the amount of strychnine contained in 1.5 c.c. extract must be

approximately nine times the minimum amount of strychnine that is detectable by the biological test (see biological test). It is therefore clear that fairly large quantities of strychnine must be present in order to render these precipitation tests of any value in the identification of strychnine. If a sufficient quantity of strychnine be present in the extract all the above precipitating agents could be used as the results of this test will give an indication as to the amount of strychnine present. Wagner's reagent No. 1 and Mayer's reagent are the most sensitive.

If large amounts of strychnine (above 5.0 mgm.) are present in the extracts to be tested the method of identifying alkaloids by precipitation described by Fulton (1930) may be found useful in confirming the evidence obtained by other chemical reactions and the biological test.

According to Seka (Klein, 1933) strychnine, weakly acidified with nitric acid, is still precipitated in the following dilutions by the undermentioned precipitants: 1:400,000 by potassium bismuth iodide, 1:100,000 by potassium mercury iodide, 1:300,000 by silicotungstic acid, 1:600,000 by phosphotungstic acid in the presence of 1 per cent. hydrochloric acid, 1:10,000-11,000 with trinitrothymol and hexanitro-diphenylamine, and 1:9,000-10,000 with picric acid. Precipitates are also formed with chlorine and bromine water. [Seka, (Klein, 1933) and Gadamer, 1924.]

#### (b) *Colour reactions for strychnine.*

(1) In 1827 Orfila (Ranke, 1879) found that an evaporated alcoholic extract of intestines to which strychnine had been added gave a red colour with nitric acid. On the other hand Gadamer (1924) and Seka (Klein, 1933) state that in concentrated nitric acid strychnine is dissolved with a yellow colour and brucine with a red colour.

(2) Cloetta (1866) states that (a) when strychnine is dissolved in concentrated sulphuric acid a violet colour appears when strong oxidising agents (e.g. potassium bichromate) are added; and (b) strychnine and chromic acid form a combination, which is almost insoluble in water. According to von Dragendorff (1879), however, strychnine chromate is *not* very insoluble in water and its precipitation can be retarded or prevented by certain foreign substances in the solution.

The sulphuric acid-potassium bichromate test, which was first proposed by Otto (Poe and Bailey, 1933) in 1846, is furthermore referred to by Ranke (1879), Witthaus (1911), Heiduschka and Meisner (1923 and 1927), Gadamer (1924), Autenrieth (1928), van Itallie en Bylsma (1928), Glaister (1931), Seka (Klein, 1933), and Poe and Bailey (1933).

If to a purified extract, as described under IV (A) a few drops of sulphuric acid be added and a small crystal of potassium bichromate be then pushed about in it with a glass rod a deep blue colour, which rapidly changes into purple, crimson and red, and then slowly fades away, results. Very similar colour reactions are obtained, if instead

of potassium bichromate (Otto's test), the following oxidising agents are used: potassium permanganate (Wenzell's test), manganese peroxide, lead peroxide, potassium chlorate and potassium iodide, potassium ferricyanide, cerium oxide (Sonnenschein's reagent) and ammonium vanadate (dissolved in concentrated  $H_2SO_4$  (Mandelin's reagent)). These colour reactions are not specified for strychnine but are also seen in all ethyl derivative of aniline and tetrahydrochinolin, provided that the position *para* to the nitrogen atom is unsubstituted [Seka (Klein, 1933) and Gadamer, 1924].

When sulphuric acid and manganese carbonate are added to strychnine a blue colour, which changes into violet and then into pink, appears.

Witthaus (1911, p. 1061) states that (1) the alkaloid *geissospermin*, contained in *Pareira brava* "behaves like strychnine with sulphuric acid and potassium dichromate; " (2) *hypaphorin*, an alkaloid obtained by Gresshof from the seeds and bark of *Hypaphorus subumbrans*, cultivated in Java as a shade-tree, and investigated by Plugge, is said to "cause a beautiful violet color, like that with strychnin" which "changes more rapidly, and soon disappears altogether with this test" and (3) "*anilin* also gives a blue-violet colour with potassium dichromate and dilute sulphuric acid, but this colour does not change to red and yellow, but to black, while a peculiar odor, somewhat resembling that of bitter almonds, is given off, which is not observed with strychnine."

According to Wormley (Poe and Bailey, 1933) *curarine* and cod liver oil give colour reactions with Otto's test similar to those seen in strychnine. He also states that a number of substances (amongst others morphine, quinine, sugar, brucine, and tartar emetic) will interfere with this test.

Fuller (Poe and Bailey, 1933) found "that the petroleum ether residues from *gelsemium* and *yohimbe* give the strychnine test"; presumably the Otto test for strychnine.

Poe and Bailey (1933) state that "Mameli made a study of the interference of certain substances employed in therapeutics on the Otto colour reaction. He found a number of drugs which more or less interfered with the test." It is unfortunate that the drugs concerned are not mentioned by Poe and Bailey as the publication of Mameli is not obtainable in South Africa.

Poe and Bailey (1933) tested a large number of organic compounds with the Otto reaction for strychnine and found a number (e.g. cryptopine, papaverine, piperine, arbutin, benzanilide, etc.), which yielded results similar to those seen in strychnine. They also found that certain organic compounds (e.g. aesculine, meta-aminophenol, azoxybenzene, benzoic acid, benzohydrol, beta-naphthol, etc.) completely covered up the Otto test for strychnine when present in equal amounts.



According to de Vry and van der Burg (Cloetta, 1866)  $\frac{1}{60,000}$  grain ( $=0.0011$  mgm.) strychnine is detectable by the Otto test. Cloetta (1866) however disagrees and states that the smallest amount of strychnine detectable by means of this test is  $\frac{1}{7,000}$  grain ( $=0.0095$  mgm.).

Gadamer (1924) states that the sulphuric acid-potassium bichromate test is still positive with  $0.001$  mgm. strychnine. Heiduschka and Meisner (1927) were able to demonstrate  $0.000125$  mgm. strychnine by means of this test, whilst Glaister (1931) referring to this test, states that "this play of colours is characteristic of strychnine, and can be perceived with the  $\frac{1}{10,000}$  grain of the poison" ( $\frac{1}{10,000}$  grain  $\pm 0.0066$  mgm.). A glance at these figures shows an enormous discrepancy.

Repeated tests conducted by the author showed only the faintest violet colour with  $0.007$  mgm. strychnine sulphate. With quantities smaller than this amount of strychnine no characteristic and reliable play of colours was obtained. The tests were conducted as follows:  $1$  gm. of strychnine sulphate was dissolved in  $1$  liter of distilled water. From this stock solution weaker solutions were prepared. Of each dilution (ranging from  $1:1,000$  to  $1:800,000$ )  $1$  c.c. was taken and evaporated on a waterbath and the residue submitted to the sulphuric acid-potassium bichromate test.

(3) Heiduschka and Meisner (1923) describe a sublimation test for strychnine. The strychnine is sublimated *in vacuo* and the following tests are then applied: (i) Precipitation with potassium bichromate by Behrens method. Strychnine bichromate is formed. (ii) Precipitation with sulphuric acid as the acid strychnine sulphate. If the sublimate is dissolved in dilute sulphuric acid, acid strychnine sulphate crystallises out in long needles. (iii) Furthermore, colour tests (Wenzel's, Mandelin's and Otto's tests) are applied.

Kempf and Eder (Gadamer, 1924, pp. 378-382) also refers to the microsublimation of strychnine.

(4) To  $4$  c.c. of a strychnine solution add an equal amount of concentrated hydrochloric acid and  $2-3$  gm. of pure granulated zinc, heat to the boiling point and leave standing for  $3-4$  minutes. If a drop of a  $1/10$  per cent. sodium nitrite solution be added to  $2$  c.c. of the above cooled solution a red colour immediately appears.  $0.003$  mgm. strychnine in  $1$  c.c. of the solution tested still yields a positive result (Malaquin-Denigès), (Gadamer, 1924).

In regard to this test Seka (Klein, 1933) adds: If to the remaining portion of the solution one to two drops of bromine water be added, a purplish-red colour appears. If more bromine water be added a precipitate which dissolves with a red-violet colour in alcohol, is formed.

(5) Erdmann's and Froehde's reagents give no colour reactions with strychnine. [Erdmann's reagent—"sulphuric acid containing nitric acid, prepared by adding to 20 c.c. of pure concentrated sulphuric acid 10 drops of a mixture of 10 drops of concentrated nitric acid and 100 c.c. of water" (Autenrieth, 1928). Froehde's reagent—7. "A solution of molybdic acid in sulphuric acid, prepared by heating gently and dissolving 5 mgm. of molybdic acid or sodium molybdate in 1 c.c. of pure concentrated sulphuric acid. The solution which should be colourless does not keep long." (Autenrieth, 1928).]

(6) According to Aloy, Valdiguié and Aloy (1926) "strychnine in  $\text{H}_2\text{SO}_4$  is unaffected by the addition of small amounts of  $\text{UO}_3$  or acetate. But on exposure to the sunlight the solution becomes violet, as a result of oxidation. This method may be used for the detection of  $1 \times 10^{-5}$  parts of strychnine."

(7) Wharton's test—"Dissolve the substance to be tested in a dry condition in chloroform. Put this solution in a small test-tube and evaporate the chloroform by setting the tube in a larger one containing boiling hot water. When the substance is dry or nearly so, add a few drops of mixture of equal parts of strong sulphuric acid and water and dissolve by shaking. Now introduce bromine vapour carefully and move the tube to and fro so that the solution takes up bromine. Replace the tube in boiling water to expel excess of bromine vapour. If strychnine is present, a carmine-red colour will appear in a few minutes, increasing in intensity as the bromine evaporates. This colour fades after a time. Instead of bromine vapour, a solution of a drop of bromine in 2 c.c. of chloroform may be used. If the quantity of strychnine present is small, only a little bromine should be added to the solution" (Autenrieth, 1928).

According to Fujiwara (1933) a reagent of sulphuric acid and sodium molybdate is specific for strychnine. No details of the tests are described in the abstract and unfortunately the Tokyo Journal of Biochemistry is not obtainable in South Africa.

### (c) *Physical and Chemical properties of Strychnine.*

The crystallography of strychnine and its salts is useful in the identification of this poison. The crystals of strychnine when combined with picric acid, picrolonic acid, p-nitro- and trinitro-benzoic acid, hydroferrocyanic acid, perchloric acid and iodic acid, are characteristic [Seka (Klein, 1933)].

According to Klobusitzky (1934) the following strychnine salts yield characteristic crystals with a 4 per cent. sodium glycerophosphate solution—sulphate, chloride, nitrate, phosphate, and the glycerophosphate.

### (C) DETECTION OF STRYCHNINE BY THE DIALYSIS METHOD.

Nunn (1932) describes a method of detecting strychnine with the use of a Graham dialyser. The specimen (organ or stomach contents) is cut into small pieces, placed in a glass jar, and then mixed with two or three ounces of water containing 2 per cent. hydrochloric

acid. The glass jar is then immersed in hot water and its contents allowed to digest for two or three hours. The contents of the glass jar are then poured into the dialyser, which is immersed in distilled water and allowed to stand for twenty-four hours. This process is repeated a second and third time, if considered necessary. The distilled water is tested with Mayer's reagent for the presence of alkaloids. If the result is negative there is no need to proceed, and if positive, the distilled water is evaporated on a water-bath to one ounce and filtered if necessary. The liquid is now made alkaline and shaken with chloroform. The chloroform is evaporated and the residue tested with sulphuric acid and potassium bichromate.

#### (D) BIOLOGICAL TESTS.

##### (a) *The Solvent.*

Before discussing the biological tests we have to consider the solvent necessary for dissolving the residue of the purified chloroform extract, which is to be injected, into white mice or frogs.

The experiment shown in Table II which was repeated twice with the same results, was conducted with physiological saline solution and distilled water, both of which were slightly acidified with sulphuric acid.

From Table II it is clear that physiological saline solution should be used as a solvent in preference to distilled water, which when injected intraperitoneally in excessive quantities may cause severe and continuous clonic spasms of the hindlegs and death. These spasms resemble, to a certain extent, those seen in strychnine poisoning in white mice.

It is not advisable to inject more than 1.5 c.c. of physiological saline solution intraperitoneally into three weeks old white mice weighing approximately 10 gm., and not more than 1 c.c. in the two weeks old white mice weighing approximately 5-6 gm.

##### (b) *The Animal.*

Hall (Ranke, 1879) was the first to recognise the importance of the "frog" in the detection of minute amounts of strychnine, especially in forensic medicine. He suggested that "frogs" be immersed in the solutions to be tested for strychnine. Harley (Glaister, 1931) modified Hall's method by injecting some of the solution to be tested into the thoracic or abdominal cavity of the frog.

Harley was able to detect 0.004 mgm. ( $= \frac{1}{16,000}$  grain) strychnine and Hall 0.013 mgm. ( $= \frac{1}{5,000}$  grain) by their respective methods.

Pickford (Ranke, 1879) produced severe tetanic spasms in "frogs" with 0.006 mgm. strychnine injected subcutaneously. Unfortunately the specific names of the frogs used are not given. According to von Rautenfeld (Weiss and Hatcher, 1922) *Rana temporaria* is unsuited to the quantitative estimation of strychnine. He found that *Rana esculenta* is 25 times more susceptible to strychnine than *R. temporaria* (Kobert, 1906). Lovett (Weiss and Hatcher, 1922) refers to the difference in susceptibility of "frogs" to strychnine.

TABLE II.  
*The effects of physiological saline solution and distilled water on white mice.*

Mouse No.	Age.	Weight in grams.	Fluid injected intraperitoneally.	Quantity of fluid injected.	Result.
1	3 weeks. ....	11	Physiological saline solution	0.5 c.c.	Negative.
2	3 weeks. ....	11	ditto	1.0 c.c.	Negative.
3	3 weeks. ....	10	ditto	1.5 c.c.	Twenty minutes after injection the animal was apathetic, breathing fairly heavily, and had a staring coat. One and a half hours after injections it appeared normal.
4	3 weeks. ....	13	ditto	2.5 c.c.	Result as in No. 3.
5	3 weeks. ....	10	ditto	3.5 c.c.	Ten minutes after injection the animal was very apathetic, breathing heavily, and had a staring coat. Two and a half hours after injection these symptoms had disappeared.
6	3 weeks. ....	10	Distilled water	0.5 c.c.	Negative.
7	3 weeks. ....	14	ditto	1.0 c.c.	Slight transient emphy.
8	3 weeks. ....	11	ditto	1.5 c.c.	Thirty minutes after injection the animal appeared very apathetic, had a staring coat and breathed heavily. Recovered overnight.
9	3 weeks. ....	11	ditto	2.5 c.c.	Twenty minutes after injection—condition as described in No. 10. Animal was still on its side (apparently paralysed) six hours after injection. Recovered overnight.
10	3 weeks. ....	10	ditto	3.5 c.c.	Ten minutes after injection the animal was breathing heavily, and had a staring coat. Whole body shivering markedly. Severe and continuous clonic spasms of hindlegs. Difficulty in walking. There was a tendency to extend the hindlegs as in strychnine poisoning. Deep and slow respiration. Not falling in convulsions when cage is knocked. Walking with hindlegs extended. Ultimately lying on left side, unable to move and showing clonic spasms of hindlegs, which are still slightly extended. Respiration became progressively slower until death occurred 40 minutes after injection.

He attributes this phenomenon to the "well-known difference in vitality of summer and winter frogs." No mention is made of the specific names of the frogs used in his experiment.

Ipsen (Weiss and Hatcher, 1922) suggests that the mouse be used in quantitative determinations of strychnine in preference to the "frog" as the latter is subject to seasonal variations in its susceptibility to strychnine.

Hatcher (Weiss and Hatcher, 1922) found that the fatal dose of strychnine sulphate for the "frog" is 0.45 mgm. per Kg. body weight, whilst according to Sollman the fatal dose is 5.5 mgm. per Kg. body weight. Kobert (1906) states that the lethal dose of strychnine injected subcutaneously into the "frog" is 2.0 mgm. per Kg. body weight. Unfortunately none of these authors mention the species of frog concerned.

It is evident from the literature consulted that frogs of the same species vary in their susceptibility to strychnine during the course of single investigations conducted over short periods of time. This is a serious disadvantage as far as the quantitative determination of strychnine by the frog-method is concerned.

The following is a summary of experiments conducted by Weiss and Hatcher (1922): (i) The common grass frog, or leopard frog (*Rana pipiens* Shreder) can be used in the quantitative estimation of strychnine after a period of fasting until the metabolism is reduced to a minimum. The period of fasting lasted about three to four weeks. Unfasted frogs are less susceptible than fasted frogs to strychnine. (ii) 0.15 mgm. strychnine sulphate per Kg. body weight induces perceptible increased reflex excitability in fasted frogs. (iii) The susceptibility of frogs to strychnine can be reduced by suitable feeding. (iv) "The removal of the liver of the frog during a period of minimal metabolism (after long fasting) has little influence on the size of the dose of strychnine required to induce increased reflex excitability. The removal of the liver during active metabolism causes an increase in its susceptibility toward small doses of strychnine so that it then behaves like a frog which had fasted for a long period, or until its metabolism was minimal." (v) "Variations in the weights of the animals within wide limits are without influence of the amount of strychnine per gram of weight required to cause increased reflex excitability."

Fühner (Autenrieth, 1928) states that "between 0.02-0.05 mgm. of strychnine nitrate is the smallest quantity capable of producing tetanic convulsions in a medium-sized frog." The exact weight of the frog and the species should have been mentioned. As the sentence stands it conveys nothing to the reader in regard to the susceptibility of the frog to strychnine.

Priestley (1930) states that either "*Rana pipiens*" or "*Rana palustris*" can be used in the quantitative determination of strychnine and that the average percentage error is 10 and rarely exceeds 15. He states that various factors (diet, temperature, etc.), have to be considered in connection with the susceptibility of frogs to strychnine.

Koll (Klein, 1933) states that tadpoles in the stage of changing from larva to frog, are very susceptible to strychnine. They show typical tetanic convulsions after subcutaneous injection of 0·0003-0·0004 mgm. strychnine. Neither species nor weights of frogs are mentioned.

According to Falck [Kofler (Klein, 1933)] young mice weighing 4-5 gm. are very susceptible to strychnine. Not more than 0·5 c.c. of the solution to be tested is injected into the skin of the back just above the tail. Quantities of strychnine as small as 0·0002 mgm. are detectable in this way. The tail is stiff and shows tremors, which are characteristic of strychnine and which can be registered on a kymograph.

As the author intended conducting experiments in order to ascertain for what period after death strychnine is detectable in carcasses, it was thought advisable to determine which animal (frog or mouse) is most suited to the biological test. Consequently a number of experiments were conducted with white mice and frogs (*Rana aqualensis* Bocagd and *Xenopus laevis* Daudin). The experiments with the white mice were repeated twelve times, and those with the frogs twice. In each case the same result was obtained. All the white mice used in these and subsequent experiments were obtained from the same breeder. This allowed of obtaining mice of a definite age and strain and all of which received the same diet. Table III embodies the result of the effects of different quantities of strychnine on three weeks old white mice.

From Table III it is evident that it is possible to detect 0·008 mgm. strychnine sulphate when it is injected intraperitoneally into three weeks old white mice weighing from 10-12 gm. It was found that fourteen day old white mice weighing about 6 gm. possess the same degree of susceptibility per unit body weight. As the latter animals weigh much less than three weeks old mice it is advisable to use the younger animals as smaller quantities of strychnine are detectable in this way.

*Rana aqualensis*\* was found to possess the same degree of susceptibility as three weeks old white mice, namely, recognisable strychnine spasms were still produced by 0·008 mgm. strychnine sulphate (injected into the dorsal lymph sac) per 10 gm. body weight of frog. The frogs varied in weight from 10-25 gm. They were very young but unfortunately their age could not be determined owing to lack of knowledge of the species, which is now being studied by Mr. Fitzsimons.

Nine times the amount of strychnine sulphate that is detectable with *Rana aqualensis* had no effect on *Xenopus laevis*. The weights of the two species of frogs used were approximately the same. All frogs were starved for sixteen days and kept under identical conditions before being used.

\* The frogs were kindly provided by Mr. White and identified by Mr. Fitzsimons, both of the staff of the Transvaal Museum, Pretoria.

TABLE III.  
*The Susceptibility of White Mice to Strychnine.*

Mouse No.	Age.	Weight in grams.	Amount of strychnine sulphate injected intraperitoneally per Kg. body weight	Result.
A.....	3 weeks.	10	0.3 mgm.	Negative.
A'.....	3 weeks.	10	0.3 mgm.	Negative.
B.....	3 weeks.	12	0.5 mgm.	Negative.
B'.....	3 weeks.	10	0.5 mgm.	Negative.
C.....	3 weeks.	10	0.7 mgm.	Negative.
C'.....	3 weeks.	10	0.7 mgm.	Negative.
D.....	3 weeks.	12	0.8 mgm.	Slight strychnine-like spasms of hind-legs, 10 minutes after injection. These were more severe when animal was excited (e.g., cage knocked). These symptoms disappeared 1 hour after injection.
D'.....	3 weeks.	10	0.8 mgm.	ditto
E.....	3 weeks.	13	1.0 mgm.	Fairly severe strychnine-like spasms 15 minutes after injection. Recovered 1 hour after injection.
E'.....	3 weeks.	10	1.0 mgm.	Severe convulsions 6 minutes after injection. Died 20 minutes after injection.
F.....	3 weeks.	10	1.5 mgm.	Died with convulsions 12 minutes after injection.
F'.....	3 weeks.	11	1.5 mgm.	Died with convulsions 15 minutes after injection.
G.....	3 weeks.	10	2.0 mgm.	Died with convulsions 11 minutes after injection.
G'.....	3 weeks.	10	2.0 mgm.	Died with convulsions 10 minutes after injection.
H.....	3 weeks.	13	2.5 mgm.	Died with convulsions 11 minutes after injection.
H'.....	3 weeks.	11	2.5 mgm.	Died with convulsions 8 minutes after injection.
I.....	3 weeks.	12	3.0 mgm.	Died with convulsions 4 minutes after injection.
I'.....	3 weeks.	10	3.0 mgm.	Died with convulsions 5 minutes after injection.
J.....	3 weeks.	12	3.5 mgm.	Died with convulsions 4 minutes after injection.
J'.....	3 weeks.	11	3.5 mgm.	Died with convulsions 3 minutes after injection.

In view of the above facts and also because (1) *Rana aqualensis* is not obtainable during autumn and winter and (2) mice react much more promptly than frogs to small amounts of strychnine (see symptoms of poisoning) it was decided to use white mice in all biological tests to be conducted. Three weeks old white mice were employed as in the determination of unknown quantities of strychnine it was at times necessary to inject 1.5 c.c. of the liquid to be tested. This volume of fluid (injected intra-abdominally) is excessive for a fourteen day old mouse weighing 5-6 gm.

#### *Symptoms of Strychnine Poisoning in White Mice.*

If amounts of strychnine slightly less than the M.L.D. be injected intra-abdominally into mice restlessness and increased excitability set in about 5-10 minutes after injection. The animals run about continuously and soon show an occasional clonic spasm of the hindlegs. The spasms increase in severity and the spasm-free interval becomes progressively shorter. Symptoms of weakness (paresis, ataxia) set in about 10-15 minutes after injection. These are soon followed by complete paralysis, the animal lying on its abdomen or side unable to move, and showing severe attacks of clonic spasms of the whole body and legs with the hindlegs rigidly extended backwards. Some affected mice "shivered" (continuous clonic spasms) continuously for more than an hour. The tail is extended upwards or in line with the body and if the animal lies on its side the front legs are extended at right angles to the body or backwards along the abdomen. The interval between the attacks depends on the severity of the case. It may vary from a fraction of a second to a few minutes. Convulsions may be brought on in spasm-free periods by lightly tapping the cage. After an attack of convulsions the respiration may stop for quite a while.

In acute cases of strychnine poisoning clonic spasms set in 2-4 minutes after injection. The symptoms are more severe than those described above and death may ensue from 5-15 minutes after injection. The head may be thrown backwards and the tail rigidly extended upwards.

In peracute cases the mouse suddenly gives a few short jumps with stiff legs and falls into convulsions with the hindlegs rigidly extended backwards and without any prodromal symptoms, a few seconds to a minute after injection. Death may occur almost instantaneously or after one or more attacks of convulsions, which follow at an interval of a second or less. In some cases the head is thrown backwards and the tail rigidly extended upwards at right angles to the body.

The rigid backward extension (in the same line as the body) of the hindlegs during attacks of convulsions is characteristic of strychnine poisoning.

#### *Symptoms of Strychnine Poisoning in Rana aqualensis.*

Young frogs weighing 18 gm., which had received 0.0144 mgm. strychnine sulphate (i.e. 0.8 mgm. per Kg. body weight) in the dorsal lymph sac, developed fairly severe tetanic convulsions fifty-five



to sixty-five minutes after injection. If left undisturbed the intervals between the convulsions varied from a few seconds to a few minutes. During these intervals the animals sat up again and appeared quite normal. If the slightest sound was made, or if the beaker containing the injected frogs was gently tapped, the animals immediately fell into convulsions again. During severe convulsions the frogs lay on their backs, the head was held up, the hindlegs were rigidly extended backwards showing continuous tetanic spasms, and the front legs were crossed. The animals recovered overnight.

Young frogs weighing 25 gm. which had received 0.05 mgm. strychnine sulphate (i.e. 2.0 mgm. per Kg. body weight) developed severe convulsions ten to twelve minutes after injection. The legs which were continuously extended backwards for an hour and a half before death, showed uninterrupted clonic spasms. Death occurred about four hours after the injection.

The symptoms of strychnine poisoning in "frogs" (species not mentioned) described by Ranke (1879) and Autenrieth (1928) are similar to those described above.

#### (E) QUANTITATIVE ESTIMATION OF STRYCHNINE.

(a) *By Weighing*.—This method is applicable only in those cases where a ponderable amount of strychnine was isolated.

(b1) *By titration of the free base with  $\frac{1}{100}$  N acid or with mercuric iodide; potassium iodide*.—Kobert (1906) refers to this method, which obviously cannot be used in the estimation of minute quantities of strychnine. Seka (Klein, 1933) states that when strychnine is dissolved in 50 per cent alcohol, it should be titrated with bromine phenol blue as an indicator. Amrhein (1934) uses methyl red as indicator and titrates with 0.02 N acid to a faint pink colour.

(b2) *By the volumetric method*.—Jonesco-Matui (1926) describes a volumetric method of determining strychnine by means of titration with 0.1 N. NaCl. According to his determinations 1.0 c.c. 0.1 N. NaCl is equivalent to 0.014 gm. strychnine. It is impossible to determine fractions of a milligram of strychnine by this method.

(c) *Biological assay*.—See (D) Biological tests.

(d) *By colour reactions*.—The intensity of the colour reactions, for example, Otto's test, is an indication of the approximate amount of strychnine present. Fairly reliable results are obtainable if the colour of the material tested is compared with a series of colours of known amounts of strychnine.

### III. FOR WHAT PERIOD AFTER DEATH IS STRYCHNINE DETECTABLE IN CORPSES AND CARCASSES.

In 1866 it was still impossible with the methods known at that time to demonstrate the presence of strychnine in the blood, organs and urine of animals and individuals that had died from strychnine poisoning (Cloetta, 1866).

It seems unnecessary to refer to all the experiments conducted in connection with the fate of strychnine in corpses and carcasses and only a few outstanding cases will be mentioned.

Thompson (Cloetta, 1866) found strychnine in the stomach of a dog four months after it had died from this poison. Taylor (Cloetta, 1866) was unable to detect strychnine in the stomach of a person, who had taken and died from the poison ten years before.

Cloetta (1866) added 1 grain ( $\approx 0.066$  gm.) strychnine nitrate to the stomachs of human beings, and placed these in glass vessels, which were closed up and buried three feet deep. After eleven and a half months he still detected strychnine in the stomach.

In 1856 Riekker (Ranke, 1879) placed the heart, lungs and liver of a bull in glass vessels and after having stirred 5 grains ( $\approx 0.33$  gm.) strychnine nitrate in solution into their contents the vessels were closed up with paper stoppers. They were then packed into a case of sawdust and stored. Eleven years and five weeks later Riekker still detected strychnine in the contents of the vessels. Ranke also refers to experiments conducted by other investigators.

Ranke (1879) killed seventeen dogs with 0.1 gm. strychnine nitrate. None of the animals vomited. Nine dogs were buried 1.5 metres deep in loose sandy soil and the remaining eight in loam soil. Carcasses were exhumed 100, 135, 200 and 330 days after burial respectively, and the specimens (stomach, intestine, liver and spleen) sent to different chemists for analysis. On the 330th day the organs were not recognisable and specimens consisting of decomposed muscle and intestines were collected for analysis. None of the chemists were able to demonstrate with any amount of certainty the presence of strychnine by means of chemical tests (colour tests) and crystallography. Biological tests conducted upon frogs however revealed the presence of strychnine even in the carcasses exhumed 330 days after burial.

Cram and Meserve (1910-1911) report on an interesting case. The body of a man, who had died from poisoning, was exhumed four months after death. "The body had been frozen most of the time but the grave when opened was full of water which was allowed to drain off. An embalming fluid of acid reaction had been used when the body was first buried, which made it appear likely that any strychnine would be dissolved out." No strychnine was obtainable from 454 gm. of the lung, 133 gm. of kidney, 446 gm. of muscle, 850 gm. of small intestine and 560 gm. of brain. 0.0015 gm. of strychnine was obtained from 803 gm. of liver and 0.0033 gm. from the spinal cord, which weighed 25 gm.

Gadamer (1924) and Autenrieth (1928) state that Kratter detected strychnine in corpses six years after death.

Ipsen (van Itallie and Bylsma, 1928) concluded from his experiments that strychnine was detectable in corpses for years after death provided there was no loss of the poison from the corpse.

TABLE IV.  
*The following dogs were drenched per stomach tube with strychnine sulphate dissolved in distilled water on the 7th September, 1933.*

No. of Log.	Apparimate Age in Years.	Weight in Kg.	Amount of strychnine sulphate (given per Kg. body weight).	Period that elapsed from time of drenching to death.	Period that elapsed from death to complete rigor mortis.
1247	4	8	5.0 mgm. (+ approximately 5 M.L.D.)	3 hours.....	35 minutes.
1263	5	14	ditto	10 minutes.....	15 minutes.
1309	5	8.5	ditto	15 minutes.....	20 minutes.
1310	6	8.5	ditto	12 minutes.....	20 minutes.
1311	6	15	ditto	10 minutes.....	20 minutes.
1312	5	16	ditto	10 minutes.....	20 minutes.
1313	5	15	ditto	11 minutes.....	15 minutes.
1314	5	15	ditto	15 minutes.....	30 minutes.
1317	6	14	ditto	20 minutes.....	20 minutes.
1318	4	11.5	ditto	15 minutes.....	30 minutes.
1319	5	12	ditto	20 minutes.....	20 minutes.
1320	6	12	ditto	12 minutes.....	10 minutes.
1321	5	9	ditto	8 minutes.....	12 minutes.
1322	5	13	ditto	Developed fairly severe spasms 1 hour after drenching. After nine attacks improvement set in, the animal appearing normal 3 hours after drenching. It was shot 4 hours after drenching	1 hour.
1323	5	13	ditto	20 minutes.....	30 minutes.
1324	6	15	ditto	25 minutes.....	30 minutes.
1325	10	31.5	ditto	13 minutes.....	20 minutes.
1326	6	23	ditto	15 minutes.....	20 minutes.
1329	5	20	ditto	10 minutes.....	15 minutes.
1330	8	23.5	ditto	12 minutes.....	20 minutes.
1331	6	18	ditto	30 minutes.....	45 minutes.
1332	4	7.5	ditto	35 minutes.....	40 minutes.
1333	4	10	ditto	10 minutes.....	15 minutes.
1334	5	7	ditto	12 minutes.....	20 minutes.

## ONDERSTEEPOORT EXPERIMENTS.

The object of these experiments was (a) to determine for what period after death strychnine was detectable in carcasses of poisoned dogs and (b) to compare ptomaines, which were isolated from control dogs, chemically (colour reactions) and biologically with the purified extracts obtained from dogs killed with strychnine.

Twenty-four dogs were killed with strychnine sulphate on the 7th September, 1933.

From Table IV it would appear that the shorter the period that elapsed between drenching with strychnine and death the sooner *rigor mortis* set in. It is of interest to note that dog 1322 survived the effects of about 5 M.L.D. of strychnine.

On the same date five control dogs (1251, 1315, 1316, 1326 and 1327) were shot.

All the dogs were placed in strong wooden boxes with lids. The boxes were placed on stones in holes five feet deep in black clay soil, and then covered with sheets of corrugated iron, supported by iron standards placed across the boxes. The layer of clay soil over the iron sheets was about three feet deep. The holes were five feet apart. At various intervals after burial dogs killed with strychnine and control dogs were exhumed and specimens collected for analysis. The dogs were then re-buried.

The method employed in the extraction of strychnine from organs and stomach contents is that described by Glaister (1931). It was, however, found that if the alcohol extract was filtered through filter paper instead of cloth (muslin) (as suggested by Glaister) the process of shaking out the alkaline aqueous extract with chloroform was much less laborious in the case of decomposed organs. In this way the possibility of the formation of an emulsion is reduced. Should such an emulsion be formed, separation of the fluids may in many cases be achieved if to the "alkaline aqueous extract-chloroform emulsion" a fair quantity of ether be added and the mixture shaken vigorously and placed alternately in an incubator at about 38° C. and in a refrigerator. If in spite of this procedure no separation of the fluids occur the only alternative is to centrifuge. In the case of decomposed carcasses (organs) the evaporated chloroform extract, which almost invariably was dirty brown in colour and had an unpleasant odour, was purified, as it was realised that only thoroughly purified extracts could be used in the colour and biological tests.

In a preliminary experiment various amounts of strychnine sulphate were dissolved in distilled water and then thoroughly mixed with minced livers. After twenty-four hours the author was able to recover 95 per cent. of the amount of strychnine added to the different specimens of liver.

From previous discussions it is clear that the least amount of strychnine required in order to apply both the potassium bichromate-sulphuric acid (Otto) test and the biological test on three weeks old

white mice is approximately 0·015 mgm., as at least 0·007 mgm. strychnine is required for the former test and 0·008 mgm. for the biological test. If 14-day old mice are used it would be possible to detect 0·012 mgm. strychnine.

As the biological assay of strychnine is much more accurate and less time consuming than a quantitative estimation by means of comparing the intensity of the colour reaction of the tested material with a series of colour reactions of known amounts of strychnine, it was decided first to apply the biological test (see next paragraph) and if sufficient material were left it was made alkaline and shaken with chloroform and the bichromate colour (Otto) test applied to the chloroform residue.

The purified extract was accordingly dissolved in 1·5 c.c. warm physiological saline solution slightly acidified with sulphuric acid. Three weeks old white mice weighing 9-12 gm. were then injected intraperitoneally with this solution commencing with 0·1 c.c. and either increasing or decreasing the dose according to whether the result was positive or negative.

From the above it is obvious that if an extract contains less than 0·015 mgm. strychnine only the biological test can be applied.

Table V embodies the results of tests conducted with the carcasses of the dogs killed with strychnine.

From Table V the following points are evident:—

(a) *Degree of decomposition of carcasses:*

- (1) Six days after death—advanced state of decomposition; skin almost hairless; organs still recognisable.
- (2) Twelve days after death—organs still recognisable.
- (3) Six weeks after death—organs still slightly recognisable.
- (4) Ten weeks after death—organs not recognisable.
- (5) Eighteen weeks after death—boxes containing the carcasses completely immersed in (and filled with) water. The boxes were continuously immersed in water for about five weeks on account of very heavy rains.
- (6) Eleven months after death: bones almost dry but still covered with a small amount of fatty substance.

(b) *Presence or absence of strychnine in carcasses.*

- (1) Three hours after death (dog 1325) strychnine was found in the lung, stomach, kidney, brain and spinal cord, spleen, muscles on posterior aspect of left femur, and the heart. Unfortunately owing to an accident the amount of strychnine in the liver could not be determined. Strychnine was found most concentrated in the stomach, lung, spleen and kidney. No strychnine was detectable in the left tibia and fibula.

TABLE V.  
*Carcasses of Dogs killed with Strychnine on 7th September, 1933.*

Dog No.	Degree of decomposition of carcass	Organs examined and amount of strychnine isolated per 100 gm. organ.
1325	<i>Three hours after death.</i> —No signs of decomposition. <i>12 days after death:</i> In advanced state of decomposition; organs still recognisable	<i>Organs examined 3 hours after death.</i> Right lung (150 gm.) : 0.1 mgm. Extract bitter. Half of liver (450 gm.) : Flask broken, hence amount of strychnine not determined. Posterior half of stomach (wall + contents = 200 gm.) : 7.5 mgm. Extract bitter. Left kidney (40 gm.) : 0.08 mgm. Extract bitter. Half of brain and spinal cord (cut longitudinally) (70 gm.) : 0.02 mgm. Extract bitter. Half of spleen (cut longitudinally) (30 gm.) : 0.1 mgm. Extract bitter. Left tibia and fibula (200 gm.) : No strychnine found. Extract not bitter. Muscles of posterior aspect of left femur (200 gm.) : 0.012 mgm. Half of heart (cut longitudinally through both ventricles = 100 gm.) : 0.016 mgm. Extract bitter.
1330	<i>Six days after death.</i> In advanced state of decomposition; skin almost hairless and very moist; organs still recognisable. <i>Eleven months after death:</i> Bones almost dry but still covered by a certain amount of a fatty substance.	<i>Organs examined 6 days after death.</i> Stomach wall—no contents present (100 gm.) : 2.4 mgm. Extract bitter. Muscles from posterior aspect of femur (300 gm.) : 0.013 mgm. Lungs (200 gm.) : 0.06 mgm. Left tibia and fibula (100 gm.) : No strychnine found. Extract bitter. Liver (200 gm.) : 0.6 mgm. Extract bitter. Heart (70 gm.) : 0.43 mgm. Extract bitter. Kidneys (75 gm.) : 0.023 mgm. Extract bitter. Spleen (30 gm.) : 0.08 mgm. Extract bitter.
1312	<i>Six weeks after death:</i> Carcass well preserved; abdominal and thoracic organs still slightly recognisable. <i>Eleven months after death:</i> Same as dog 1247.	<i>Organs examined 6 weeks after death.</i> Abdominal contents (400 gm.) : 0.3 mgm. Extract bitter. Bones (both femurs and skull) (300 gm.) : 0.04 mgm. Extract bitter.
1328	<i>Six weeks after death:</i> Carcass very decomposed; bones already exposed. <i>Eleven months after death:</i> Bones almost dry but still covered by a certain amount of a fatty substance.	<i>Organs examined 11 months after death.</i> Bones (almost dry) (400 gm.) : No strychnine detectable. Animal showed slight transient apathy after injection. Extract not bitter.
		<i>Organs examined 11 months after death.</i> Bones (almost dry) (350 gm.) : 0.005 mgm. Extract has a slight bitter taste.

# DETECTION OF STRYCHNINE IN CARCASSES AND CORPSES.

Dog No.	Degree of decomposition of carcass.	Organs examined and amount of strychnine sulphate isolated per 100 gm. organ.
1313	<i>Ten weeks after death</i> : Only skeleton, which fairly dry, and hair left. <i>Eleven months after death</i> : Same as dog 1247.	<i>Organs examined 10 weeks after death.</i> Bones (skull and left tibia and fibula) (270 gm.): 0 09 mgm. Extract bitter.
1314	<i>Ten weeks after death</i> : Only skeleton, which is fairly dry, and hair left.	<i>Organs examined 10 weeks after death.</i> Bones (skull and left tibia and fibula) (300 gm.): No Strychnine detectable.
1311	<i>Eleven months after death</i> : Same as dog 1247. <i>Ten weeks after death</i> : Only skeleton, which is fairly dry and hair left. <i>Eleven months after death</i> : Same as dog 1247.	<i>Organs examined 10 weeks after death.</i> Bones (skull and left tibia and fibula) (300 gm.): 0 04 mgm. Extract bitter.
1310	<i>Ten weeks after death</i> : Only skeleton, which is fairly dry, and hair left. <i>Eleven months after death</i> : Same as dog 1247.	<i>Organs examined 10 weeks after death.</i> Bones (skull and left tibia and fibula) (180 gm.): 0 13 mgm. Extract bitter.
1247	<i>Eighteen weeks after death</i> . Box full of water. Decomposed matter floating on water in box : bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry. Bones almost dry but still covered by a certain amount of a fatty substance.	<i>Organs examined 18 weeks after death.</i> Bones (skull and left tibia and fibula) (120 gm.). No strychnine detectable. The injected mouse ran about in an excited manner for about 3 minutes and showed ataxia, but no spasms. Recovered 30 minutes after injection. Extract not bitter.
1309	<i>Eighteen weeks after death</i> . State as described in dog 1247.	<i>Organs examined 18 weeks after death.</i> Bones (skull and left tibia and fibula) (400 gm.): 0 005 mgm. Extract not bitter.
1253	<i>Eighteen weeks after death</i> : State as described in dog 1247.	<i>Organs examined 18 weeks after death.</i> Bones (skull and left tibia and fibula) (200 gm.). The symptoms exhibited by the mouse injected with the total quantity of extract (1.5 c.c.) exhibited symptoms which were not very typical of strychnine. Hence a toxic plasma may be the cause. Extract is tasteless.
1317	<i>Eighteen weeks after death</i> : State as described in dog 1247.	<i>Organs examined 18 weeks after death.</i> Bones (skull and left tibia and fibula) (172 gm.): Symptoms exhibited were not very typical of strychnine. Extract not bitter.

Twelve days after death strychnine was found less concentrated in the stomach and more concentrated in other organs. This was due to the fact that strychnine diffused through the decomposed stomach wall thus permeating the whole carcass. This explains the phenomenon that no strychnine was detectable in the left tibia and femur collected three hours after death, whilst twelve days later the poison was detectable in the right tibia and femur.

It is of interest to note that no strychnine was detectable in the left tibia and fibula of dog 1330 six days after death, whilst the other organs contained large quantities of strychnine.

- (2) Six weeks after death strychnine was still detectable in the "abdominal contents" and bones of dogs 1312 and 1328.
- (3) Ten weeks after death strychnine was present in the bones of dogs 1313, 1311 and 1310, whilst it could not be detected in the bones of dog 1314.
- (4) Eighteen weeks after death strychnine was present in the bones of dog 1309, whilst it was not detectable in the bones of dogs 1247, 1253, and 1317. It should be mentioned that the carcasses were found immersed in water at the time when the specimens were collected. It is quite conceivable that the strychnine present in the bones was dissolved out by the water. The less "fatty" the bones are the greater the likelihood of this happening.
- (5) Eleven months after death the bones of dogs 1330, 1328, 1313, and 1314 still contained strychnine whilst the poison was not detectable in the bones of dogs 1312, 1311, 1310 and 1247. It is of interest to mention that a number of the extracts prepared from the bones of dogs killed with strychnine had a bitter taste in spite of the fact that no strychnine was detectable in them.

#### **IV. DISCUSSION AND RECOMMENDATIONS AS TO THE MOST RELIABLE METHOD OF DIAGNOSING STRYCHNINE POISONING.**

##### **A. METHOD OF EXTRACTING STRYCHNINE.**

The method described by Glaister (1931) yields most satisfactory results. A high degree of purification of the extracts prepared from organs in an advanced state of decomposition can be achieved by taking up the evaporated chloroform extract in a few c.c. of distilled water acidified with sulphuric acid and shaking this out with a small quantity of ether. The aqueous solution is then rendered alkaline with potassium hydroxide and again shaken out with chloroform. This process should be repeated if necessary. In this way the salts of ptomaines, which are soluble in ether will be removed. This is an advantage as some ptomaines (see IV. D. Ptomaines) are chemically (colour reactions) and biologically almost indistinguishable from strychnine.



Weiss and Hatcher (1922) found that "the Stas-Otto method for the extraction of poisons from animal tissues does not permit of the recovery of strychnine quantitatively when only very small amounts (but such as may be present exceptionally at the time of death) are present, but widely diffused in the organs." They proposed the liquefaction of tissues with 20 per cent. sodium hydroxide and heat and then shaking out with chloroform.

As stated before the author obtained most satisfactory results with the method used by himself.

Priestley (1930) described a method of isolating strychnine from blood and organs, which according to him is less time-consuming and as accurate as methods used by other investigators.

## B. THE DETECTION OF STRYCHNINE *intra vitam* AND IN CORPSES AND CARCASSES

### (a) *Intra vitam*.

In cases of acute strychnine poisoning the vomit (if vomition occurs) and the urine should be collected and tested for the presence of strychnine. The method of extraction described by Weiss and Hatcher (1922) could be used.

Weiss and Hatcher state that "the kidneys excrete amounts equal to 20 per cent. of that administered at one time, and a much lower percentage of larger doses taken by the mouth over periods of twelve and twenty-eight hours, respectively. The percentage of the strychnine excreted by the kidneys is a measure of the eliminative efficiency of the liver, rather than that of the kidney itself, for the kidney excretes only that which the liver *fails* to excrete.

Diuresis hastens the elimination of strychnine by the kidney, but it does not necessarily increase the total amount eliminated in the urine after a single dose injected intramuscularly, and it may, in fact, be attended with the renal elimination of a smaller total than would occur in a similar experiment without diuresis."

According to Gadamer, (1924) 50-75 per cent. of the strychnine, administered in large but non-lethal doses, is excreted by the kidneys.

From the literature it appears that no strychnine is detectable in the urine a few days after administration, the greatest proportion of the amount eliminated in the urine being excreted within the first few hours.

Strychnine is not detectable in the faeces (Autenrieth, 1928).

### (b) *A Short Period after Death.*

The amount of strychnine recoverable from the corpse or carcass depends on the following circumstances.

(1) *The dose administered and the method in which it was administered.*

Large doses of strychnine cause death within a few minutes or hours, especially when administered subcutaneously or intravenously,

as there is little time for elimination in the urine, bile, milk, saliva, etc. In the case of large doses with death soon after the poison had been taken per os a high percentage of the amount taken will be found in the stomach contents provided no vomition occurred.

In regard to the absorption of strychnine from the stomach Ryan (1912-13) found that strychnine nitrate in alcoholic solutions (10 to 20 per cent. alcohol) is not absorbed as readily as in aqueous solution. He also found that strychnine is fairly readily absorbed by the gastric mucous membrane.

If a dose of strychnine equivalent to the M.L.D. or slightly more, had been administered no strychnine, or mere traces, will be detectable in the stomach contents. The comparatively long period that elapses between administration and death allows of complete, or almost complete, absorption of the poison from the gastrointestinal tract.

If the M.L.D. of strychnine for the human being be calculated on the generally accepted basis of 1.0 mgm. per Kg. body weight i.e.  $\frac{1}{1,000,000}$  part of the body weight) a human being weighing 70 Kg. will require 0.07 gm. (approximately 1 grain) of strychnine to cause death. If it be assumed that the strychnine taken is evenly distributed throughout the body, and that no excretion or destruction of the poison has occurred, then the corpse will contain 0.1 mgm. strychnine per 100 gm. This amount of strychnine will allow of precipitation and colour tests and the biological test being conducted.

It appears, however, that strychnine is not evenly distributed in corpses and carcasses but that apart from the stomach contents, which should be analysed, the greatest proportion of strychnine is to be found in the liver, spleen, kidneys, lung and brain and spinal cord [Autenrieth (1928) and others]. In cases of acute and peracute strychnine poisoning the urine (if any is present in the corpse or carcass) should also be analysed. When decomposition of corpses and carcasses sets in the strychnine, which is present in the stomach, will diffuse through the stomach wall and permeate the other organs.

Weiss and Hatcher (1922) experimenting upon cats found that "strychnine sulphate leaves the blood stream rapidly, and after two minutes as much as 30 per cent. may have left the circulation; within five minutes more than 50 per cent. and after forty minutes the blood may contain only about 4 per cent. of that injected." It therefore appears that the blood is not a suitable specimen for the isolation of strychnine.

As strychnine has cumulative effects it is quite possible that small amounts of strychnine may be detected in the body (especially liver, spleen, lungs, bones, and central nervous system) of persons, and animals receiving strychnine as a tonic for a certain period before death due to some cause other than strychnine poisoning.

In cases of suspected strychnine poisoning where the following information is at our disposal we may be able to discriminate between

cases, which have received strychnine as a tonic and have died from some other cause, and cases of strychnine poisoning:—

- (i) The evidence of the doctor or veterinary surgeon, who attended the patient or animal concerned;
- (ii) The symptoms of poisoning and post-mortem appearances;
- (iii) The amount of strychnine present in the gastro-intestinal tract and in the organs.

In the case of a person or animal receiving strychnine as a tonic the drug will be completely absorbed within a few hours after administration. It is, however, possible that strychnine may be detectable in minute quantities in the gastrointestinal contents and wall in cases which received strychnine as a tonic, owing to the fact that the drug is partially excreted in the gastrointestinal tract and in the bile.

It is obvious that the weight of the specimens analysed for the presence of any poison is of the utmost importance. The heavier the specimen the more reliable the results will be.

The author conducted the following experiment (see Table VI) in order to ascertain whether strychnine taken as a tonic, that is, in very small (non-toxic) amounts, is detectable in carcasses or not.

From Table VI it is evident that strychnine was detectable in the liver and stomach of two dogs, which had received non-toxic amounts of strychnine daily for twenty-two days, and which were killed three hours after administration of the last dose of strychnine. It is apparent that a very large proportion of the amount of strychnine administered in the last dose had at the time of death (3 hours after administration) already disappeared from the stomach wall and contents. The kidneys of dog 1438 showed an advanced degree of cirrhosis. No strychnine was detectable in the organs of dog 1408, which was killed three days after administration of the last dose of strychnine. Sufficient time had evidently elapsed for the excretion and destruction of the poison.

The detection of strychnine in carcasses and corpses of individuals and animals, which had received a tonic containing strychnine and which died from a cause other than strychnine poisoning, depends on (1) the amount of strychnine administered, (2) the period that elapsed between the last dose and death, (3) the period that elapsed between death and the time of analysis of the corpse or carcass, (4) the age of the individual or animal, (5) the state in which the organs of excretion (especially liver and kidneys) were, (6) whether or not embalming fluids were used, and (7) on the rainfall.

It is interesting to note that the purified extracts prepared from the lungs and heart of dogs 1438 and 1439 contained a paralytic poison(s).

(2) *The treatment applied.*—It is obvious that the amount of strychnine present in corpses and carcasses especially in the stomach depends on the treatment administered to the victims. A certain proportion of the strychnine present in body will be removed or destroyed by (i) stomach lavage, (ii) emetics, and (iii) oxidising agents (e.g. potassium permanganate).

TABLE VI.  
*Strychnine toxic Experiment.*

Dog No.	Weight.	Amount of strychnine sulphate administered.	Killed on.	Result of analysis of organs and remarks.
1408 (aged).	22.8 Kg.	0.066 mgm.* per Kg. body weight daily from 28.2.34 to 24.3.34	27.3.34	At no time did the animal exhibit any symptoms of poisoning. No strychnine was detectable in the following organs: Lungs and heart (300 gm.), liver (500 gm.), spleen (40 gm.), stomach wall plus contents (500 gm.), brain and spinal cord (100 gm.), and kidneys (80 gm.).
1438 (aged).	22.5 Kg.	0.1 mgm.* per Kg. body weight daily from 11.4.34 to 2.5.34	2.5.34	At no time did the animal exhibit any symptoms of poisoning. Killed three hours after administration of the last dose of strychnine. (1) Brain and spinal cord (80 gm.): No strychnine detectable. (2) Lungs and heart (400 gm.): The white mouse, which was injected with the extract, developed pronounced apathy and paresis, but recovered after two hours. (3) Liver (500 gm.): 0.025 mgm. strychnine sulphate present. (4) Spleen (30 gm.): No strychnine detectable. (5) Stomach wall and contents (700 gm.): 0.015 mgm. strychnine sulphate present.
1439 (6 years old)	16 Kg.	0.1 mgm.* per Kg. body weight daily from 11.4.34 to 2.5.34	2.5.34	At no time did the animal exhibit any symptoms of poisoning. Killed three hours after administration of the last dose of strychnine. (1) Brain and spinal cord (80 gm.): No strychnine detectable. (2) Lungs and heart (250 gm.): No strychnine detectable. The white mouse, which was injected with the extract, developed symptoms of paralysis and died two hours after injection. (3) Liver (400 gm.): 0.016 mgm. strychnine sulphate present. (4) Spleen (30 gm.): No strychnine detectable. (5) Kidneys (50 gm.): No strychnine detectable. (6) Stomach wall and contents (500 gm.): 0.014 mgm. strychnine sulphate present.

\* 0.2 gm. strychnine sulphate was dissolved in 1,000 c.c. distilled water and the requisite amount administered.

Tannic acid will combine with the strychnine still present in the gastrointestinal tract forming the insoluble tannate.

(3) *Destruction of Strychnine by the Body Tissues and Fluids intra vitam.*—According to Autenrieth (1928) the largest proportion of strychnine administered is destroyed “in the living organism as a result of animal metabolism”.

(c) *After the Corpses and Carcasses are in a state of advanced decomposition.*

In perusing the literature (see III) dealing with the persistence of strychnine in corpses and carcasses it appears that this poison is very resistant to the processes of decomposition. In some cases strychnine was detected a few years after death. These records should, however, be regarded with a critical eye as the methods used in the detection of strychnine are open to criticism.

In many cases only taste and colour tests (Otto's test, Mandelin's test) were used whilst other investigators applied the biological test only. In the discussion on ptomaines (IV. D) it will be seen that ptomaines resembling strychnine, either chemically or biologically are known. It has already been stated that both the colour and biological tests for strychnine should be applied in order to come to definite conclusions.

The amount of strychnine present in the body at the time of death may in the course of time decrease in the following ways:—

(1) *Method of Burial or Disposal of Corpse or Carcass.*—It is obvious that cremation completely destroys the poison. Embalming retards processes of decomposition, hence it is probable that strychnine will persist in such corpses for longer periods than in unembalmed corpses. The possibility of the embalming fluid destroying, or chemically changing strychnine, should also be considered.

Low temperatures inhibit processes of decomposition, hence the possibilities of detecting strychnine in corpses and carcasses buried in ice- and snow-ridden areas are more favourable than in warm areas.

A certain amount of strychnine escapes with the fluid (decomposed blood and liquefied tissues) flowing out of the corpse or carcass. It is therefore obvious that in cases in a state of advanced decomposition specimens of the garments and coffin (unless tin-lined) should also be taken as these were saturated with the fluid referred to. If there is evidence of the fluid having percolated through the coffin specimens of the soil concerned should also be analysed. In the case of tin-lined coffins containing bones only it is advisable to thoroughly rinse out the coffins with a small quantity of 96 per cent. alcohol acidified with acetic acid.

In the case of carcasses, which are decomposed it is essential that specimens of underlying soil be analysed for strychnine. Carcasses are sometimes covered with quicklime, which cause rapid disintegration of the tissues with consequent loss of fluid which soaks into the underlying soil.

The author conducted a number of experiments in order to determine whether processes of decomposition have a destructive effect on strychnine. Bovine livers were minced, placed in tins and definite amounts of strychnine (dissolved in water) added. These tins were stored at room temperature during summer. After a month practically no decrease in the amount of strychnine added had occurred, whilst after four months only about 1/50 of the original amount of strychnine added was present.

It was noticed that according to the method of biological assay (intraperitoneal injections into three weeks old white mice) much larger amounts (6 to 10 times) of strychnine were detectable in the extracts prepared from the decomposed livers if these extracts were not thoroughly purified before injection. This phenomenon was most probably due to the presence of ptomaines, which have actions similar to those of strychnine. We also have to consider the possibility of ptomaines, which have effects opposite to those of strychnine, being present in the extracts examined. In this case the biological test may not reveal the presence of strychnine in spite of the fact that it is present in the extract.

In one case the extract prepared from a control liver (to which no strychnine was added), which was analysed after having been allowed to decompose for four months, yielded a positive potassium bichromate-sulphuric acid test, which was indistinguishable from that of strychnine.

(2) *Rainfall.* It is obvious that the presence of water in graves will dissolve out some of the strychnine present in corpses provided they are not enclosed in coffins (tin-lined and sealed) to which water has no access. In the case of corpses treated with acid embalming fluids large amounts of, if not all, the strychnine present in the corpse will be dissolved and removed by the water as strychnine salts are easily soluble in water. The less decomposed the carcass the less strychnine will be dissolved by the water as the fatty substances present will to a certain extent prevent the entrance of water into the organs and bones.

In the Onderstepoort experiment it was found that the boxes containing the carcasses of the twenty-nine dogs were floating in water after about 12 in. of rain had fallen in less than five weeks. A month later, after another 6.28 ins. of rain had fallen, the boxes were still submerged. It was interesting to note that after 6 inches of rain had fallen in the course of three weeks the soil (black clay) was wet only about 18 inches deep and there was no water in the holes.

#### (C) THE MOST SUITABLE TESTS FOR THE DETECTION OF STRYCHNINE.

It has already been stated that for reasons mentioned above the taste test and at least one colour test (Otto's or Mandalin's test, etc.), and the biological test should be employed for strychnine before a definite opinion can be expressed. The author achieved very satisfactory results with the Otto test in conjunction with a biological test conducted upon three weeks old white mice.

The greater the number of tests for strychnine the more reliable the results will be.

#### (D) SUBSTANCES RESEMBLING STRYCHNINE.

The substances resembling strychnine chemically (colour tests, etc.), and biologically could be discussed under (a) chemical substances other than ptomaines, and (b) ptomaines.

##### (a) Chemical Substances other than Ptomaines.

(i) Kobert (1906), Witthaus (1911) and Gadamer (1924) refer to a substance (pellagrozoin) which is sometimes present in decomposing maize and which resembles strychnine both chemically and biologically.

(ii) The colour reactions seen when oxidising agents are added to strychnine are not specific for this drug, but are also seen in all ethyl derivatives of aniline and tetrahydrochinolin, provided that the position *para* to the nitrogen atom is unsubstituted [Seka (Klein, 1933)]. Methyl- and ethyl-strychnine, which differ in action from strychnine, also give a positive Otto test.

(iii) The following alkaloids when injected in comparatively large amounts also induce tetanus in frogs: brucine, thebain, morphine, hydrastine and caffeine [Kofler (Klein, 1933)].

(iv) Brucine, quinine, corchorin, picric acid, and many other substances also have a bitter taste.

(v) Poe and Bailey (1933) studied the colour reactions of the Otto test on a large number of chemical substances and found twenty-three compounds which gave some shade of violet, lavender, or purple. Some of the colours seen were very similar to those given by strychnine. The alkaloids, cryptopine and papaverine, gave violet colours, so also did phenylglocine (amino acid derivative). Allylphenylthiocarbamide gave a brown colour with streaks of violet and the glucosides, aesculin and arbutin, brown with a trace of violet and a reddish violet colour respectively. Furthermore, the following substances gave traces of, or, a distinct violet colour: aniline derivatives + ortho- anisidine, benzanilide, ortho-benzotoluide, para-bromoaniline, ortho-phenetidine, para-phenetidine; ortho-amino-phenol; anisic acid (aromatic acid); naphthalene and anthracene derivatives + alizarin, alpha-naphthylaminoazobenzene, miscellaneous aromatic compounds: benzylphenylhydrazine, tetrabromophenolphthalein, and triphenylmethane.

(vi) The alkaloids *getssospermin* and *hypaphorin* are said to cause a colour reaction like that seen in strychnine with sulphuric acid and potassium bichromate.

##### (b) Ptomaines.

Ptomaines (animal alkaloids, putrefactive alkaloids, cadaveric alkaloids) are defined as bases "formed under the action of bacteria or of metabolism by the splitting of carbon dioxide from an amino-acid."

In 1865 Aeby and Schwarzenbach extracted from corpses substances which induced tetanus (Kobert, 1906). Selmi prepared from corpses a substance which resembled strychnine chemically (Kobert, 1906). Kobert also states that Maas isolated from muscle and brain in the first stages of decomposition a ptomaine which caused tetanus; and that Anthor (see also Gadamer, 1924, p. 593) found a ptomaine which resembled strychnine chemically.

Ranke (1879) refers to ptomaines isolated from carcasses in a state of advanced decomposition which have a narcotic (paralytic) effect on frogs. He correctly remarks that the presence of such ptomaines masks the pharmacological effects of strychnine.

According to Witthaus (1911) Baumert states that "in one case the ptomaine in question not only gave various chemical reactions, including the identifying reactions of strychnine, but also possessed the tetanizing action of that alkaloid". Unfortunately no particulars about this case are available.

Mecke isolated a faintly bitter, non-tetanizing ptomaine which gave a positive Otto test and was coloured yellow by Erdmann's reagent, and dirty violet by Fröhde's reagent (Witthaus, 1911; Gadamer, 1924).

Gadamer (1924) groups the ptomaines according to their solubility in organic solvents. He remarks that the ptomaines which are capable of being dissolved by chloroform out of an alkaline solution are said to have a pungent bitter taste and that they reduce iodic acid to iodine. "They are also said to give a red colour with sulphuric acid and Fröhde's reagent?"

According to Kippenberger many ptomaines mentioned in the literature are nothing else but peptones (Gadamer, 1924).

#### *Onderstepoort Experiments with Ptomaines.*

(i) *Unidentified ptomaines isolated from the carcasses of dogs killed as controls in the strychnine experiment.*—Whenever carcasses of the dogs killed with strychnine were exhumed, specimens for analysis were also collected from the carcasses of the control dogs which were shot. The extracts from the organs were prepared by the same method which was used in the isolation of strychnine. It is therefore obvious that only those ptomaines, which have the same solubility characters as strychnine are concerned in Onderstepoort experiments. When organs in an advanced state of decomposition are analysed for the presence of strychnine it is advisable to take up the chloroform residue in a small quantity of acidulated ( $\text{H}_2\text{SO}_4$ ) distilled water and then shake out once with ether. The ether, in which strychnine sulphate is practically insoluble, will remove from the aqueous solution those ptomaines which are soluble in this organic solvent.

Table VII embodies the results of the extracts prepared from the organs of the control dogs.



TABLE VII.  
*Control Dogs—Shot on 7 September, 1933.*

Dog No.	Approximate age.	Weight in Kg.	Degree of decomposition of carcass.	Effects of extracts of organs injected intraperitoneally into three weeks old white mice.
1326	5 years	28	<p><i>Six days after death:</i> (carcass very decomposed; skin very moist and almost hairless; internal organs very decomposed but still recognisable.</p> <p><i>Eighteen weeks after death:</i> Box full of water. Decomposed matter floating on water in box; bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry.</p>	<p><i>Liver:</i> 0.1 c.c. extract (16.6 gm. liver) caused death in a mouse 5 minutes after injection. No spasms. Animal collapsed and died.</p> <p><i>Spleen:</i> 0.1 c.c. extract (166.6 gm. liver) induced a few clonic spasms of the hind-legs. The legs were, however, not rigidly extended backwards as in strychnine poisoning. Extract not bitter.</p> <p><i>Stomach contents:</i> 0.1 c.c. extract (13.3 gm. stomach wall and contents) induced no symptoms in a mouse.</p> <p><i>1.0 c.c. extract (133.3 gm. stomach-wall and contents)</i> induced clonic spasms of whole body closely resembling those seen in strychnine poisoning. Death occurred 10 minutes after injection. Extract not bitter.</p> <p><i>Spleen:</i> 0.1 c.c. extract (2 gm. spleen) induced no symptoms in a mouse.</p> <p><i>0.1 c.c. extract (20 gm. spleen)</i> induced pronounced dyspnoea, collapse and death 12 minutes after injection. No spasms. Extract not bitter.</p> <p><i>Bone (tibia and fibula and marrow):</i> 0.1 c.c. extract (6.6 gm. bone) induced no symptoms in a mouse.</p> <p><i>1.0 c.c. extract (66.6 gm. bone)</i> induced no symptoms in a mouse. Extract not bitter.</p> <p><i>Lung:</i> 0.1 c.c. extract (6.6 gm. lung) induced no symptoms in a mouse.</p> <p><i>1.0 c.c. extract (66.6 gm. lung)</i> induced collapse and death 4 minutes after injection. The lungs were stiff and for breath but showed no spasms. Extract not bitter.</p> <p><i>Kidney:</i> 0.1 c.c. extract (6.6 gm. kidney) induced collapse and death without spasms 6 minutes after injection.</p> <p><i>1.0 c.c. extract (66.6 gm. kidney)</i> induced collapse and death without spasms 2 minutes after injection. Extract not bitter.</p> <p><i>Heart:</i> 0.1 c.c. extract (6.6 gm. heart) induced dyspnoea, clonic spasms of whole body and death in a mouse 20 minutes after injection. The hind-legs, however, not extended backwards as in strychnine poisoning.</p> <p><i>1.0 c.c. extract (66.6 gm. heart)</i> induced death in a mouse 3 minutes after injection. The head was thrown backwards but hind-legs not extended backwards as in strychnine poisoning.</p> <p><i>Marrow of posterior aspect of humerus:</i> 0.1 c.c. extract (16.6 gm. marrow) induced no symptoms in a mouse.</p> <p><i>1.0 c.c. extract (166.6 gm. marrow)</i> induced no symptoms in a mouse. Extract not bitter.</p> <p><i>Organs examined 18 weeks after death:</i> <i>Bone (right tibia and fibula plus vertebrae):</i> N.B. Extract had a slightly bitter taste. 0.1 c.c. extract (10 gm bone) induced no symptoms. 1.0 c.c. extract (100 gm. bone) induced repeated clonic spasms of hind-legs and of body 10 minutes after injection. The convulsions closely resembled those seen in strychnine poisoning.</p> <p><i>Extract of a slight bitter taste.</i></p>

Dog No.	Approximate age.	Weight in K.g.	Degree of decomposition of carcass.	Effects of extracts of organs injected intraperitoneally into three weeks old white mice.
1316	4 years	19 Kg.	<i>Six weeks after death:</i> Carcass in advanced state of decomposition; nothing left of internal organs; <i>Eighteen weeks after death:</i> Box full of water. Decomposed matter floating on water in box; bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry.	Organs examined 6 weeks after death. <i>Hair and remains of skin:</i> 0.1 c.c. extract (10 gm. hair and skin) induced no symptoms in a mouse. <i>1 0 c.c. extract (200 gm. bone):</i> Induced dyspnoea in a mouse; 4 minutes after injection the animal jumped into the air, fell down on left side, and showed spasms of the hindlegs very much resembling those seen in strychnine poisoning. The hindlegs were, however, not as rigidly extended backwards as in strychnine poisoning. Death occurred 7 minutes after injection. Extract not bitter. <i>Bone (skull and femur):</i> 0.1 c.c. extract (20 gm. bone) induced no symptoms in a mouse. <i>1 0 c.c. extract (200 gm. bone):</i> Induced dyspnoea in a mouse 4 minutes after injection. Then signs of all 4 legs. The animal made short quick jumps for about 5 minutes. Then showed spasms of hind legs resembling those seen in strychnine poisoning. The animal died 10 minutes after injection. Extract not bitter.
1315	6 years	23 Kg.	<i>Six weeks after death:</i> Slightly less decomposed than dog 1316. Internal organs not recognisable. <i>Eleven months after death:</i> Bones almost dry but still covered by a certain amount of fatty material.	Organs examined 11 months after death. <i>Bones (almost dry):</i> 1 0 c.c. extract (270 gm. bone) induced no symptoms of paralysis and gasping for breath three minutes after injection. Death occurred 5 minutes after injection. No spasms were seen. Extract bitter. Otto's test slightly positive.
1251	5 years	19 Kg.	<i>Ten weeks after death:</i> Only skeleton, which is almost dry, and hair left. <i>Eleven months after death:</i> Bones almost dry but still covered by a certain amount of fatty substance.	Organs examined 11 months after death. <i>Bones (almost dry):</i> 1 0 c.c. (170 gm. bone) induced transient symptoms of paresis and laboured respiration. Two hours after injection the animal appeared normal again. Extract not bitter.
1327	6 years	18 Kg.	<i>Ten weeks after death:</i> Only skeleton, which is still fairly moist, and hair left. <i>Eleven months after death:</i> Bones almost dry but still covered by a certain amount of fat.	Organs examined 11 months after death. <i>Bones (almost dry):</i> 1 0 c.c. extract (240 gm. bone) induced laboured respiration and symptoms of paresis 15 minutes after injection. The animal gave continuous short jumps and was completely paralysed when it died 30 minutes after injection. Extract slightly bitter.

The following is evident from Table VII.

- (1) Dog 1326—the extract prepared from the stomach (plus contents) collected six days after death induced symptoms in white mice very closely resembling those seen in cases of strychnine poisoning. The extract was, however, not bitter and gave a negative Otto test.

The extract prepared from the bones collected eighteen weeks after death also induced strychnine-like spasms. The extract had a slight bitter taste and yielded a negative Otto test.

- (2) Dog 1316—the extract prepared from the bones collected six weeks after death induced strychnine-like spasms. The Otto test was negative and the taste not bitter.
- (3) Dog 1315—the extract prepared from the “abdominal contents” exhumed six weeks after death yielded strychnine-like spasms and a negative Otto test and was not of a bitter taste.

The extract of the bones exhumed eleven months after burial induced no strychnine-like spasms. The result of the Otto test very much resembled that seen in strychnine poisoning and the extract also had a bitter taste.

- (4) Dog 1251—the extract prepared from the bones exhumed ten weeks after death induced strychnine-like spasms. The Otto test was, however, negative and the taste of the extract not bitter.
- (5) Dog 1327—the extract of the bones exhumed ten weeks after death induced strychnine-like spasms and the taste was intensely bitter. The Otto test was, however, negative.

The extract of the bones exhumed eleven months after death induced no strychnine-like spasms. The extract was slightly bitter in taste but yielded a negative Otto test.

The remaining extracts had no chemical or biological characters which could be mistaken for those of strychnine.

A number of the extracts contained narcotic ptomaines.

(ii) *Identified ptomaines.*—The undermentioned ptomaines were obtained from Messrs. Fraenkel and Landau, Berlin-Oberschöne-weide, Germany, and were submitted to the following tests. (Table VIII.)

For the colour test 0.2 gm. of the above ptomaines was used. For the biological test the amounts of ptomaines mentioned in the above table were dissolved in 0.5-1.0 c.c. physiological saline solution (sterilised) and injected intraperitoneally into three weeks old white mice.

Not in a single case did the result of the colour tests resemble the play of colours seen in strychnine. On the other hand neurin-bromide, guanidine carbonate, cholin chloride (0.001 gm.) and trimethyl-pyridine induced spasms which resembled those seen in cases of strychnine poisoning.

Cadaverine and putrescine in the doses mentioned above appear to be typical paralytic poisons for white mice.

Promaine.	Potassium chromate-sulphuric acid test	Biological test (conducted upon 3 weeks old white mice).
(1) Neurinbromide (colourless crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of a few small crystals of potassium bichromate a dark brown colour, which changes to a dark green, appears.	<i>Mouse No. 1 (7 gm.)</i> : 0.0125 gm. neurinbromide. The animal collapsed and died a few seconds after injection. Spasms slightly resembling those of strychnine-like poisoning were seen. The hindlegs were, however, not very rigidly extended backwards. <i>Mouse No. 2 (8 gm.)</i> : 0.005 gm. neurinbromide. Same result as above. <i>Mouse No. 3 (9 gm.)</i> : 0.001 gm. neurinbromide. Convulsions of the whole body were seen but the hindlegs did not get so rigidly extended. Death occurred one minute after injection. <i>Mouse No. 4 (10 gm.)</i> : 0.0002 gm. neurinbromide. No symptoms appeared.
(2) Guanidine carbonate (small white crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of a few crystals of potassium bichromate a dark yellow colour, which then became slight greenish, appeared.	<i>Mouse No. 5 (9 gm.)</i> : 0.05 gm. guanidine carbonate. Three minutes after injection the animal became very excited, and showed extremely laboured respiration. It made movements of the hindlegs and forelegs. After a further three minutes it fell into convulsions, extending the hindlegs and forelegs. Death occurred after injection. <i>Mouse No. 6 (10 gm.)</i> : 0.025 gm. guanidine carbonate. The symptoms described in <i>Mouse No. 5</i> set in five minutes after injection. Death occurred seven minutes after injection.
(3) Cadaverine dihydrochloride (small white crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of potassium bichromate a yellow colour, which changed to a dark green, appeared.	<i>Mouse No. 7 (10 gm.)</i> : 0.025 gm. cadaverine dihydrochloride. Two minutes after injection the animal walked stiffly. Dyspnoea and paralysis (no spasms) then set in. Two hours after injection the animal appeared to be dead. <i>Mouse No. 8 (8 gm.)</i> : 0.05 gm. cadaverine dihydrochloride. Progressive paralysis and dyspnoea set in a few minutes after injection. No spasms were seen. Death occurred three hours after injection. <i>Mouse No. 9 (10 gm.)</i> : 0.1 gm. cadaverine dihydrochloride. The animal was completely paralysed three minutes after injection. It made no movements and no spasms were seen. Death occurred about two and a half hours after injection.
(4) Cholin chloride (small white crystals)	The crystals were dissolved in concentrated sulphuric acid and upon the addition of a few potassium bichromate crystals a yellow colour, which changed into green, appeared.	<i>Mouse No. 10 (10 gm.)</i> : 0.025 gm. cholin chloride. Within a few seconds after injection the animal was paralysed; and it fell into convulsions and died almost at once. No strychnine-like spasms were seen. <i>Mouse No. 11 (10 gm.)</i> : 0.005 gm. cholin chloride. The results were the same as in <i>mouse No. 10</i> . <i>Mouse No. 12 (10 gm.)</i> : 0.001 gm. cholin chloride. Symptoms of paresis (weakness) set in five minutes after injection. After a further ten minutes the animal was paralysed and showed strychnine-like spasms of the hindlegs.
(5) Cholin (base). (Brownish fluid)	The fluid was mixed with a few drops of concentrated sulphuric acid and a few crystals of potassium bichromate added. The result was a dark green colour.	<i>Mouse No. 13 (7 gm.)</i> : 0.065 gm. cholin. Death occurred three minutes after injection after the animal had exhibited laboured respiration (gasping for breath), and symptoms of paralysis. No spasms were seen. <i>Mouse No. 14 (10 gm.)</i> : 0.013 gm. Cholin. Symptoms very similar to those described in <i>mouse No. 13</i> set in 2 minutes after injection. In addition, there were clonic spasms of the whole body and legs. There were, however, no spasms, which resembled those seen in strychnine poisoning. <i>Mouse No. 15 (10 gm.)</i> : 0.0026 gm. cholin. No symptoms of poisoning were seen.
(6) Putrescine dihydrochloride (white crystalline powder)	The crystals dissolved in concentrated sulphuric acid with effervescence. Upon the addition of potassium bichromate a dark colour appeared, which changed to a green, appeared.	<i>Mouse No. 16 (9 gm.)</i> : 0.025 gm. putrescine dihydrochloride. The animal died with symptoms of progressive paralysis twenty minutes after injection. No spasms were seen. <i>Mouse No. 17 (9 gm.)</i> : 0.15 gm. putrescine dihydrochloride. Died with symptoms of progressive paralysis fifteen minutes after injection.
(7) 2-4-6 - Trimethylpyridine (colourless fluid)	A few drops of concentrated sulphuric acid added to the fluid caused the formation of a white precipitate, which soon redissolved, forming a colourless liquid. Upon the addition of a few crystals of potassium bichromate to this colourless liquid a dark green colour appeared.	<i>Mouse No. 18 (9 gm.)</i> : 0.065 gm. trimethylpyridine. Within a few seconds after injection the head was thrown back violently and repeatedly. There were clonic spasms of the hindlegs very closely resembling those seen in strychnine poisoning. Death occurred half-a-minute after injection. <i>Mouse No. 19 (10 gm.)</i> : 0.013 gm. trimethylpyridine. Death occurred half-a-minute after injection. The symptoms were seen. <i>Mouse No. 20 (9 gm.)</i> : 0.004 gm. trimethylpyridine. Death occurred one minute after injection the animal collapsed with clonic spasms of the hindlegs, resembling those seen in strychnine poisoning. <i>Mouse No. 21 (10 gm.)</i> : 0.0008 gm. trimethylpyridine. A few minutes after injection dyspnoea and symptoms of paresis set in. The hindlegs were extended backwards, but not as rigidly as in strychnine poisoning. The hindlegs were slightly extended backwards, but not as rigidly as in strychnine poisoning. Death occurred twenty-seven minutes after injection.

## V. SUMMARY.

(1) In order to express a definite opinion as to the presence or absence of strychnine in *purified* extracts of specimens of organs, etc., it is essential that the following tests be conducted: (a) taste test, (b) colour test, and (c) a biological test. Immature white mice are for various reasons more suited to the biological test than frogs, (*Rana esculenta*, *Rana pipiens*, *Rana palustris*, *Rana aqualensis*).

It is definite that very *unreliable* and *inaccurate* results will be obtained if both the colour and biological tests for strychnine are not applied to extracts as a large number of chemical substances, including ptomaines, are known which yield positive results either with the colour test, or with the biological test for strychnine. Many of these substances also have a bitter taste. The greatest care should be exercised in expressing an opinion as to the presence of strychnine in decomposed carcasses and corpses. The author isolated a strychnine-like ptomaine(s) from a decomposed liver, which was known not to contain any strychnine. This ptomaine(s) had a bitter taste and gave a positive sulphuric acid-potassium bichromate test for strychnine. The results of taste, chemical and biological tests with unidentified and identified ptomaines are recorded.

(2) If three weeks old white mice are used in the biological test at least 0.008 mgm. strychnine sulphate is required in order to produce recognisable strychnine spasms in a mouse weighing 10 to 12 gm. With 14 day old white mice weighing 5 to 6 gm. 0.004 mgm. strychnine sulphate is detectable. In order to achieve reliable results in the detection of strychnine in *purified* extracts of organs, etc., at least 0.011 mgm. strychnine sulphate should be present as approximately 0.007 mgm. is required for the Otto test and 0.004 mgm. for the biological test if this is conducted upon 14-day old white mice weighing from 5 to 6 gm. If three-weeks-old white mice are employed the least amount of strychnine detectable in extracts is 0.015 mgm. if both the Otto and biological tests are conducted.

(3) The symptoms of strychnine poisoning in white mice and in the frog (*Rana aqualensis*) are described.

(4) The taste test and chemical and biological tests for strychnine are discussed.

(5) Factors responsible for the disappearance of strychnine from corpses and carcasses are discussed. Of four dogs killed with strychnine and exhumed ten weeks after death strychnine was detectable in three carcasses, whilst of four carcasses exhumed eighteen weeks after death only one was positive for strychnine. Eleven months after death eight carcasses of dogs killed with strychnine were exhumed and strychnine was detectable in only four of these. Subsequent exhumations of carcasses of dogs killed with strychnine and of control dogs are to be conducted.

(6) Methods of extracting strychnine from carcasses and corpses and of purifying these extracts are discussed.

(7) In fresh carcasses and corpses the most suitable organs for analysis for the presence of strychnine are liver, stomach, spleen, lung and the central nervous system; also the urine.

(8) In two out of three dogs, which had received strychnine as a tonic, strychnine was detectable in the liver and stomach (plus contents).

(9) A large number of chemical substances, which resemble strychnine chemically and biologically, are discussed.

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## Section VII.

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# Mineral Metabolism and Nutrition.

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- VAN WYK, C. M., BOTHA, M. L., AND BEKKER, J. G. The effect of supplements of different forms of sulphur to the diet upon the wool of merino sheep.
- MALAN, A. I., DU TOIT, P. J., AND GROENEWALD, J. W. Studies in Mineral Metabolism XXXIII. Iodine in the nutrition of sheep (2nd Report).
- DU TOIT, P. J., MALAN, A. I., LOUW, J. G., HOLZAPFEL, C. R., AND ROETS, G. A study of the mineral content and feeding value of natural pastures in the Union of S.A. (Third Report).
- DU TOIT, P. J., LOUW, J. G., AND MALAN, A. I. A study of the mineral content and feeding value of natural pastures in the Union of S.A. IV. The influence of season and frequency of cutting on the yield persistency and chemical composition of grass species.





## **The Effect of Supplements of Different Forms of Sulphur to the Diet upon the Wool of Merino Sheep.**

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By

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### **INTRODUCTION.**

CONSIDERABLE prominence has lately been given to the effect of adding sulphur supplements to the diet of sheep. Whatever the results may be from a nutritional or physiological point of view, the possible effect on the wool, as regards both quality and total production, naturally assumes importance, and should be investigated. This aspect was first remarked upon by Steyn (1931, 1932), who concluded that a sulphur supplement in the diet had a beneficial effect on the total production of wool. Subsequently Seddon (1933) recorded that he had found no significant effect on the wool yield of sheep, entirely grazed, while Pierce (1933) found no beneficial effect on sheep kept on a maintenance ration, or one slightly above maintenance.

The following are the results of an investigation of the wool of sixty Merino wethers which had received supplements of sulphur in various forms. The influence on the body weights of the same sheep has been dealt with in another publication (du Toit, Malan, Groenewald and Botha, 1935), where it was shown that, under the conditions of the experiment, the supplements had no significant effect on the body weight as compared with the control sheep. For this and other aspects of the question, it is only necessary to refer to the above publication, where full details will be found.

### **EXPERIMENTAL DETAILS.**

#### **A. SHEEP AND MANAGEMENT.**

A group of seventy-five Merino wethers, born at Grootfontein between 10.3.1930 and 9.6.1930, were selected for uniformity of type, fleece, and body weight. They were shorn between 31.3.1931 and 8.4.1931, and subsequently sent to Onderstepoort, where they arrived on 8.10.1931 as two-tooths. Here they were shorn in December, 1931, and sixty of them placed on a basal ration for eleven

months. During this (pre-experimental) period all the sheep received identical treatment, but were divided into six uniform groups and placed in a gravelled paddock with pens and boxes for individual feeding. At the conclusion of this period (November, 1932), they were shorn again and placed on the same basal ration for a period of twelve months, during which the sheep in five of the groups were dosed with various forms of sulphur, the sixth group acting as a control. They were finally shorn in November, 1933. For brevity the two periods will be called the 1932 and 1933 periods respectively. The conditions under which the wool was grown may thus be summarised as follows:—

1932: Eleven months on basal ration.

1933: Twelve months on basal ration and supplements.

### B. FEED.

The basal ration throughout the two periods 1932 and 1933 consisted of half a pound of crushed yellow maize and half a pound of green feed—lucerne or maize stalks or maize ensilage during about three months in winter, when no green feed was available. In addition the animals had free and continual access to teff hay. For further details of the method of feeding and the consumption, as also of the general care and management of the sheep, the reader is referred to the paper quoted above (du Toit *et alia*, 1935).

During the experimental period (1933) the sheep were dosed daily except Sundays, the following being the supplements given to each animal:—

Group I: No supplement.

Group II: 0.45 gm. cystine.

Group III: 0.7 gm. of a mixture consisting of 0.2 gm.  $K_2SO_4$ , 0.3 gm.  $MgSO_4$ , and 0.2 gm.  $CaSO_4$ .

Group IV: 0.36 gm. KCNS.

Group V: 5.0 gm. sulphur.

Group VI: 0.12 gm. sulphur.

The quantities of supplements, except in the case of Group V, were calculated to contain the same amount of sulphur as that contained in the 0.45 gm. cystine given to each animal in Group II.

### C. MATERIAL.

Immediately before shearing in December, 1931, a wool sample of about 15 gm. was cut from the shoulder of each sheep. A pair of fine scissors was employed, and the wool cut as close to the skin as possible. The area of about one and a half inches square, from which the sample had been cut, was then tattooed on the skin, and at subsequent shearings the wool growing on this area was first removed. In addition a further sample was taken adjacent to this area for chemical analysis, which is still in progress. This method ensured that the samples obtained at the end of successive periods were taken from the same area on the skin of each sheep.

The samples having been removed, the sheep were shorn and the fleeces stored in linen bags for examination.

#### D. METHODS OF ANALYSIS.

(a) The grease weight, clean weight and yield percentage of the fleece.

At the time of shearing each fleece was properly skirted in the manner customary in shearing sheds. The skirtings were transferred to a smooth-topped table and again worked through carefully—heavy dung-locks were discarded, while all the lightest pieces that would blend with the fleece were run in with the latter. Fleeces and locks, individually, were then transferred to suitable linen bags and stored in a non-ventilated room. Subsequently these were weighed under identical conditions, due allowance being made for the weights of the shoulder samples which had been removed for physical and chemical analysis.

For the determination of the clean weights and yield percentages of individual fleeces, a modification (Botha) of the method described by Wilson (1928) was followed. The method may be summarised as follows:—

- (1) Subsequent to the weighing referred to above, each fleece was passed three times through a fleece-breaking machine, the object being to blend the various parts of the fleece thoroughly in order to enable representative samples to be drawn from it.
- (2) The fleece was weighed again to allow for impurities lost during the process of blending and for probable fluctuations in moisture content.
- (3) Immediately after this weighing two samples of a hundred grams each were made up of small amounts of wool, of approximately twenty grams, taken at random from various parts of the bulk of the fleece.
- (4) These samples were next scoured in a scouring liquor consisting of a mixture of potassium oleate soap and sodium carbonate.
- (5) After being allowed to become air-dry, the air-dry weights of the samples, and of a standard sample of known dry weight—in hygroscopic equilibrium—were recorded.
- (6) Immediately after this a sub-sample of twenty grams of air-dry wool was taken from the last-scoured sample of each fleece and dissolved in 200 c.c. N/NaOH solution (i.e. 4 per cent.), in order to determine the amount of vegetable matter present after scouring. The clean dry weights of the fleeces could then be calculated and converted to clean conditioned weight at 17 per cent. regain (International regain). The clean conditioned weight of wool contained in the locks was obtained separately in a slightly modified manner.

Next the air-dry grease weight of the locks was added to that of the corresponding fleece, and similarly the clean conditioned weight. From the resulting totals the weighted mean of the conditioned yield percentage of each total fleece was calculated.

• The grease and clean conditioned weights given below represent the mean production over twenty-four hours.

The conditioned yield percentage is defined as the conditioned clean weight of the fleece expressed as a percentage of the original air-dry grease weight of fleece prior to machine treatment.

NOTE.—All grease weights have been expressed on an air-dry basis irrespective of actual moisture content. A higher or lower moisture content of the grease wool at the time of the initial weighing would be reflected merely in a lower or higher yield percentage. Such differences in the actual moisture content of the greasy wool could be brought about (*a*) by differences in atmospheric temperature and humidity in the different years at the time of weighing individual fleeces and (*b*) by the possibility that when the fleeces were stored in a closed non-ventilated room, equilibrium of moisture content may not have been attained.

It must be emphasised, however, that, by the nature of the calculations, the result for the conditioned clean weight remains totally unaffected by fluctuations of the moisture content of the greasy wool, no matter how wide these may have been. Even in the case of yield percentages, differences will be of the order of a few per cent. only, since the actual fluctuations in humidity will be comparatively small.

(*b*) Mean length, thickness and fibre weight of a sample.

The weight-length method (Roberts, 1930), was used for the analysis of the samples. It consists briefly of (1) a method of sampling, (2) the counting of a number of fibres, (3) the measurement of the lengths of a certain proportion, and (4) the determination of the dry weight of the fibres counted. From the data thus obtained the following are calculated:—

Mean length :  $l$ .

Mean thickness :  $\sqrt{\frac{4W}{\pi \times 1.3 \times n \times l}}$ .

Mean fibre weight :  $\frac{W}{n}$ ,

where  $W$  = dry weight of the counted fibres,

$n$  = number of fibres counted,

$l$  = mean length of one-tenth of the counted fibres,

1.3 = specific gravity of dry wool.

In addition, a check on the sampling and on the accuracy of the result is obtained in the form of a coefficient of fineness dispersion. It should be pointed out that Roberts expresses his results as fibre fineness, which is the length, in centimetres, of fibre which weighs

one milligram. The thickness given in this paper is the diameter of the fibres calculated on the assumption that they are uniform circular cylinders.

The samples were divided into ten approximately equal zones, and ten sub-samples made up, each consisting of one small lock from each zone. These sub-samples therefore collectively consisted of a hundred small locks taken from different points in such a way as to represent the whole of the marked area. Each sub-sample was analysed separately, so that ten different estimates of the length, fineness and fibre weight of the original sample were obtained. About two thousand fibres in all were counted from each of the pre-experimental samples, and four thousand from each of the experimental samples, the coefficients of fineness dispersion ranging from one to four per cent. The length of every tenth fibre was measured, and the dry weights were determined by comparison with a standard sample of known dry weight, the weighings being repeated daily for several days in succession.

The microscopical method of measuring thickness (Deurden, 1929), was applied to a number of the samples. This method gave essentially the same results, though the values were all somewhat higher; when routine analysis have to be made the authors are inclined to favour the microscopical method, especially in the case of Merino wool, where the fibres are very fine. A further comparison of the two methods, involving measurements on about a hundred samples, will be reserved for a later publication.

## RESULTS AND STATISTICAL ANALYSIS.

Below are given the group means for the two periods, 1932 and 1933. A method of analysis of variance and co-variance (Fisher, 1932), has been employed to test the significance of group differences. It is realised that in connection with wool a very large proportion of the variation between different sheep under similar conditions is due to what may be described in general terms as individuality, and is independent of normal nutritional treatment. This part of the variance of values of the experimental period may be regarded as having been foreshadowed in the values of the pre-experimental period. The appropriate coefficient of linear regression is given by the ratio of the co-variance to the variance of the independent variate, which in this case is the variance in the preliminary values.

(a) Grease weights of the fleeces, in gm. calculated for one day's growth.

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.....	10·7	10·7	9·6	10·8	9·9	10·8	10·4
1933.....	14·2	14·3	12·9	14·0	13·7	14·0	13·8

# EFFECT OF SULPHUR ON THE WOOL OF MERINO SHEEP.

The sums of squares and products are given in the following table.

$x_2$  = Grease weight in 1932.

$x_3$  = Grease weight in 1933.

	Degrees of freedom.	$x_2^2$	$x_2x_3$	$x_3^2$
Between groups.....	5	12.51	10.25	10.92
Within groups.....	47	73.43	76.55	124.03
TOTAL.....	52*	85.94	86.80	134.95

\* There was one value missing in each of the two years. These were supplied by calculating the best-fitting value from the data for three years (Yates, 1933). This accounts for the fact that there is one degree of freedom less.

The result of applying the correction for the regression, as explained above, and analysing the variance of the adjusted yields, may be derived directly from the analysis of sums and products already presented. If  $b$  stands for the regression coefficient, comparisons of the adjusted values will be comparisons of quantities  $(x_3 - bx_2)$ . Now

$$(x_3 - bx_2)^2 = (b^2x_2^2 - 2bx_2x_3 + x_3^2).$$

In the present example,

$$b = 1.0425$$

$$-2b = -2.0850$$

$$b_2 = 1.0868$$

and applying this directly to the sums of squares and products above, the following analysis is obtained.

Analysis of adjusted grease weights:—

	Degrees of freedom.	Sums of squares.	Mean squares.
Between groups.....	5	3.1446	0.6289 = $V_1$
Within groups.....	46*	40.973	0.8907 = $V_2$
TOTAL.....	51	44.1176	—

The ratio  $R = V_1/V_2$  is used to find the significance of the difference between the two variances (Snedecor, 1934). In the present case,

$$R^{-1} = \frac{8907}{6289} = 1.416,$$

$$n_1 = 46,$$

$$n_2 = 5,$$

which is very insignificant and does not indicate any differential effect between the treatments.

(b) Clean weights of the fleeces in grams, calculated for one day's growth.

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.. .. .	4 76	4 86	4 53	4 94	4 60	5 23	4 82
1933.. .. .	6 47	6 53	6 26	6 59	6 55	6 86	6 55

The same procedure has been followed in analysing the clean weights, with the following results:—

$y_2$  - clean weight for 1932.

$y_3$  - clean weight for 1933.

#### *Sums of Squares and Products.*

	Degrees of freedom.	$y_2^2$	$y_2y_3$	$y_3^2$
Between groups.....	5	2 8718	1 9453	1 6702
Within groups.....	47	17 8063	16 8198	30 2822
TOTAL.....	52	20 6781	18 7651	31 9525

$$b = 0.9446.$$

#### *Analysis of Adjusted Values.*

Variance.	Degrees of freedom.	Sum of Squares.	Mean Squares.
Between groups.....	5	0.5576	0.1115
Within groups.....	46	14.3942	0.3129
TOTAL.....	51	14.9518	---

$$R^{-1} = 2.806.$$

$$n_1 = 46.$$

$$n_2 = 5.$$



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There is again no significant difference between the variances and consequently there is no reason for assuming that the clean weights were affected by the sulphur supplements.

(c) Yield per cent. of the fleeces:—

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.....	44.5	45.7	47.4	45.9	46.5	48.1	46.3
1933.....	45.5	45.6	48.7	47.3	48.0	49.2	47.4

From the above analysis it is clear that no significant value as regards yield per cent. can be expected.

$z_2$  = yield per cent. for 1932.

$z_3$  = yield per cent. for 1933.

## Sums of Squares and Products.

	Degrees of freedom.	$z_1^2$	$z_2 z_3$	$z_3^2$
Between groups.....	5	77.52	85.23	107.31
Within groups.....	47	841.27	555.30	601.50
TOTAL.....	52	918.79	640.53	708.81

$$b = 0.6601.$$

## Analysis of Variance of Adjusted Values.

Variance.	Degrees of freedom.	Sums of squares.	Mean squares.
Between groups.....	5	28.5733	5.5147
Within groups.....	46	234.946	5.1075
TOTAL.....	51	263.5193	—

$$R = 1.0797.$$

$$n_1 = 5.$$

$$n_2 = 46.$$

As was expected, the variance between groups is not significantly different from that within groups.

(d) *Mean fibre length in cm. calculated for 30 days' growth.*

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.....	0.791	0.809	0.820	0.824	0.829	0.820	0.814
1933.....	0.909	0.944	0.968	0.981	0.954	0.955	0.950

*Analysis of Variance.* $l_2$ —1932 length,  $l_3$ —1933 length.*Sums of Squares and Products.*

	$l_2^2$	$l_2 l_3$	$l_3^2$
Between groups.....	7,866 3	10,769.1	25,635 0
Within groups.....	122,135 4	118,446 8	192,028 6
TOTAL.....	130,001 6	129,215 9	217,663 6

$$b = 0.9939.$$

*Analysis of Adjusted Values.*

Variance.	Degrees of freedom.	Sums of squares.	Mean squares.
Between groups.....	5	11,998.5	2,399.7
Within groups.....	39	74,307 6	1,905 3
TOTAL.....	44	86,306 1	—

$$R = 1.259.$$

$$n_1 = 5, \quad n_2 = 39.$$

In the case of length, therefore, this insignificant value shows that the variance between groups does not differ from that within groups.

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(c) *Mean fibre thickness in  $\mu$  (weight-length method).*

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.....	15.65	15.85	15.29	15.82	14.93	15.59	15.52
1933.....	17.66	17.86	17.13	17.90	17.22	17.56	17.55

*Analysis of Variance.*

$t_2$ —1932 thickness,  $t_3$ —1933 thickness.

*Sums of Squares and Products.*

	$t_2^2$	$t_2 t_3$	$t_3^2$
Between groups.....	4.5405	3.8621	4.0138
Within groups.....	46.4337	42.2156	53.9736
TOTAL.....	50.9742	46.0777	57.9874

$$b = 0.9094.$$

*Analysis of Adjusted Values.*

Variance.	Degrees of freedom.	Sums of squares.	Mean squares.
Between groups.....	5	0.7443	0.1488
Within groups.....	39	15.5721	0.3993
TOTAL.....	44	16.3164	—

$$R^2 = 2.683.$$

$$n_1 = 39, \quad n_2 = 5.$$

Here the same result is obtained as in the case of all the attributes studied, viz., no significant difference between groups.

(f) Mean fibre weight in  $10^{-6}$  gm., calculated for 30 day's growth.

Group.	I.	II.	III.	IV.	V.	VI.	Mean.
1932.....	1.98	2.09	1.96	2.09	1.89	1.98	2.00
1933.....	2.91	3.07	2.89	3.17	2.90	2.90	2.97

#### *Analysis of Variance.*

$w_2$  = 1932 weight,  $w_3$  = 1933 weight.

#### *Sums of Squares and Products.*

	$w_2^2$	$w_2w_3$	$w_3^2$
Between groups.....	22 165	29 595	51 901
Within groups.....	359 795	349 533	630 623
TOTAL.....	381 960	379 128	682 524

$$b = 0.9715.$$

#### *Analysis of Adjusted Values.*

Variance.	Degrees of freedom.	Sums of squares.	Mean squares.
Between groups.....	5	15 318	3.0636
Within groups.....	39	291 059	7.4630
TOTAL.....	44	306 377	—

$$R^2 = 2.436.$$

$$n_1 = 39, \quad n_2 = 5.$$

Here again as in all the previous cases there is no suggestion of any response to the sulphur supplements, as regards mean fibre weight of the samples.

It will be noted that in all cases the variance between groups does not differ significantly from that within groups. One is therefore led to the conclusion that under the conditions of the experiment, neither the fleece as a whole nor the fibres of a shoulder sample show any indication of having been affected by the supplements.

### **SUMMARY AND CONCLUSIONS.**

(1) Six groups of young wethers were kept on a production ration for two years. During the second year five of the groups received supplements of sulphur and of compounds containing

## EFFECT OF SULPHUR ON THE WOOL OF MERINO SHEEP.

sulphur, the sixth group acting as control. The grease weights, clean weights, and yield percentages of the fleeces, and the mean fibre lengths, mean fibre thickness, and mean fibre weights of shoulder samples were determined.

(2) The sheep were fed in individual feeding boxes, except for the hay, which was given *ad lib.*

(3) The following supplements were dosed daily, except Sundays:

- Group I: No supplement.
- Group II: Cystine.
- Group III: Sulphates.
- Group IV: KCNS.
- Group V: 5 gm. sulphur.
- Group VI: 0.12 gm. sulphur.

(4) There was no significant difference between the groups, showing that there was no response to any of the supplements, as regards the above-mentioned wool attributes.

### ACKNOWLEDGMENT.

The authors are indebted to Mr. A. P. Malan, M.Sc., statistician at Onderstepoort, for the statistical analysis of the results.

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## **Studies in Mineral Metabolism XXXIII.**

### **Iodine in the Nutrition of Sheep.**

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#### **SECOND REPORT.**

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By

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#### **INTRODUCTION.**

IN 1932 a two-year experiment carried out at this Institute on the effect of .02 gm. Potassium Iodide daily per head in the ration of three groups of ewes kept on different levels of phosphorus intake was reported on. The iodide was apparently without effect on the growth, food consumption, wool growth and mortality. In regard to reproduction detrimental effects were noticed during the second lambing season, 16 months after the beginning of the investigation in the groups receiving the iodide and the inference was made that a daily dose of .02 gm. KI had a detrimental effect in 1931. Mention was made of the possibility that a smaller dose of KI might not have produced harmful effects and that a new experiment had been started in which progressively larger doses of KI were given to different groups of ewes to investigate the matter of size of dosage and its effect. The latter experiment is reported on in this publication.

A study of the literature reveals that iodized stock licks are still widely recommended by investigators who claim to have obtained good results from Iodine feeding (Mason 1933, Weiser and Veghelyi 1932, Klein 1933, Wendt 1931, Potter *et al* 1931, Scharrer 1933, etc.), although it should also be mentioned that several research

workers have not been able to obtain beneficial effects (Crichton 1931, Malan, du Toit, and Groenewald 1932, Lines 1933, Forbes, Karns *et al* 1932, etc.). Some of these latter workers suggest that the indiscriminate use of iodine in stock licks may even affect stock detrimentally; Crichton concludes that our present state of knowledge does not warrant the general practice of feeding supplementary iodine to cows. As a careful survey of the literature strongly suggests that reports of adverse results of iodine feeding are, generally speaking, on the increase, the above conclusion of Crichton should, in the opinion of the writers, be made to apply to stock generally and even to poultry. While evidence against iodine feeding is accumulating, practical agriculturists would be wise to pause in order to consider whether the inclusion of iodine in their supplementary feeds is worth the extra cost of this product. It should however be mentioned, as was done in the first publication (Malan, du Toit, and Groenewald, 1932), that the continuous use of small quantities of iodine in stock licks or feeds has not been demonstrated conclusively to be detrimental to the health or production of stock, and that this and the hope of producing beneficial effects are probably the main causes of the continued use of iodine in stock licks and supplementary feeds.

By iodine feeding is meant the inclusion of iodine or its compounds in licks and feeds of livestock as advised by many enthusiasts without consideration of the existence of an iodine deficiency, under which conditions, as for instance in areas where endemic goitre occurs, it seems more than reasonable to expect beneficial results from the addition of iodine to the daily ration of animals. Most of the work done on this problem dealt with the effect of iodine feeding without regard to the existence of an iodine deficiency and was undertaken with the intention of finding out whether or not any extra benefit will accrue if iodine is given to stock apart from the question whether extra iodine over and above that which the constituents of the ration contain, is required. No attempt is therefore made to keep the iodine content of the ration low, which obviously might vary for different countries and may explain partly the differences in the results obtained by different workers as stated in the earlier publication (Malan *et al* 1932).

It is interesting to note that the chemical analyses of foodstuffs do not always confirm the existence of an iodine deficiency in goitrous areas (Ucko 1932, Blom 1933) and strengthens the view that endemic goitre cannot always be attributed to iodine deficiency *per se* (Ucko 1932, Olesen 1933). However, low iodine in foods is usually associated with the occurrence of goitre.

#### DESCRIPTION OF EXPERIMENT.

Forty uniform two-tooth merino ewes were selected and divided into four groups. These animals were kept in a small gravelled paddock 60 ft. by 90 ft. Individual feeding pens were built in an

adjoining paddock where the animals were allowed to take their concentrates and green feed during the early afternoon and overnight. During the rest of the day they were kept in the larger paddock mentioned above. Across this paddock is a shed for shelter and a hay rack about 60 feet long, where the ewes had free access to hay regularly replenished. Drinking water was always available. The green feed and the concentrates were given in separate wooden boxes in the individual feeding pens. Food consumption was registered by weighing back at intervals the feed left over, although the ration was such that it was almost invariably completely consumed as stated later in this article. The sheep were weighed at monthly intervals, shorn four times in the course of the experiment, inspected daily, kept practically free from internal parasites throughout the course of the experiment and dipped against keds on a few occasions. The fleeces were kept for differential study, and observations on oestrus were made regularly. No attempt was made to exercise the sheep and this was considered unnecessary in view of the frequent disturbance of the sheep at weighing time, feeding, oestrus and general inspection.

### *Feeding.*

All the sheep were given the same basal ration, viz.—

225 grms. crushed yellow maize;

225 grms green feed.

Hay *ad lib.*

The maize and green feed were given daily in small separate wooden boxes in the individual feeding pens. Teff hay was given in a rack *ad lib.* and the total consumption recorded, from which it appeared that each sheep consumed on an average 2 lb. of hay daily. The KI was given in solution by adding it to the maize which, as already stated, was consumed without exception. The stock solution of KI contained 10 grms. KI per 5,000 c.c. solution and was given to the groups as follows:—

Group I—controls—received no KI solution.

Group II received 1 c.c. KI solution, i.e. .002 gm. KI.

Group III received 10 c.c. K.I. solution, i.e. .02 gm. K.I.

Group IV received 30 c.c. KI solution, i.e. .06 gm. KI.

The ration was adequate for growth and the sheep remained in prime condition except during lactation when insufficient protein was present and consequently a rapid drop in weight took place. This statement is confirmed by a glance at the weight curves of the sheep.

The green feed consisted of green lucerne, or green maize or barley or even silage depending on the green feed available, which was cut and then given. The green feed was not always readily



eaten but the total quantity left over a period was negligible. For instance, the maximum amount of green feed left by an animal for the whole period March, 1932, to October, 1934, was about 10 kg. out of the total of 164 kg. given and hence a complete record of the green feed left is omitted from this publication. In any case the green feed was included in the basal ration not on account of its feeding value, i.e. the nutrients it contained but to eliminate vitamin A deficiency, the effects of which might interfere with the course of the experiment or at all events can easily be made to appear in sheep and cattle kept on a dry ration for a period (du Toit *et al* 1935).

Practically all the green feed that was not eaten was left during the rainy period October, 1933, until April, 1934. The feeding boxes were not protected against rain and the green feed was invariably left untouched and was weighed back as "not eaten" after precipitation. The average quantity of green feed left per sheep per group during the experiment was as follows:—

Group I—controls: 4.5 kg.

Group II—receiving .002 gm. KI: 4.4 kg.

Group III—receiving .02 gm. KI: 5.6 kg.

Group IV—receiving .06 gm. KI: 4.4 kg.

These figures may be worthy of record but the group differences are certainly insignificant.

### *Weighing.*

The records of the monthly weights of the four groups of sheep are given in figures I, II and III. The weight curve of the control group is given in each figure for comparison.

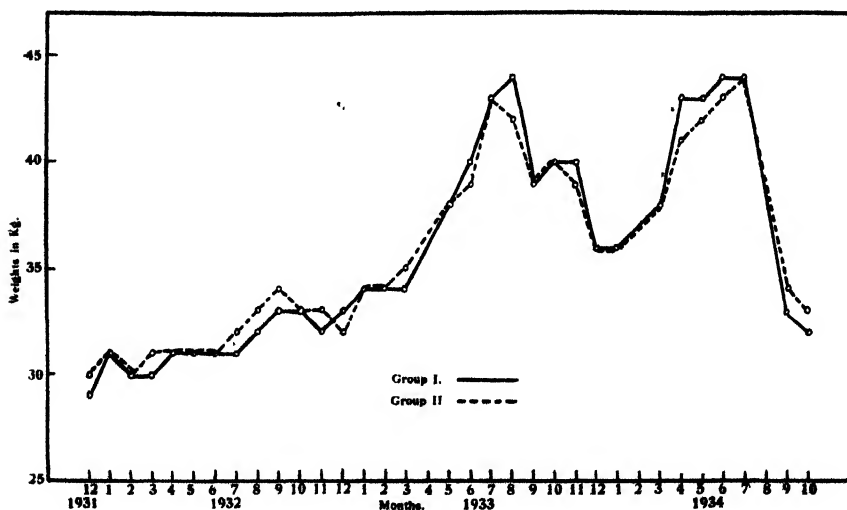


Fig. I.—Monthly Weights of Sheep.

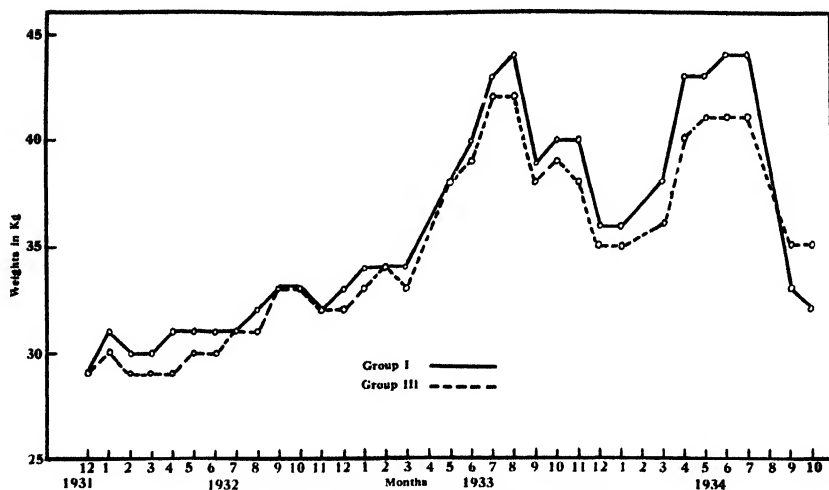


Fig. II.—Monthly Weights of Sheep.

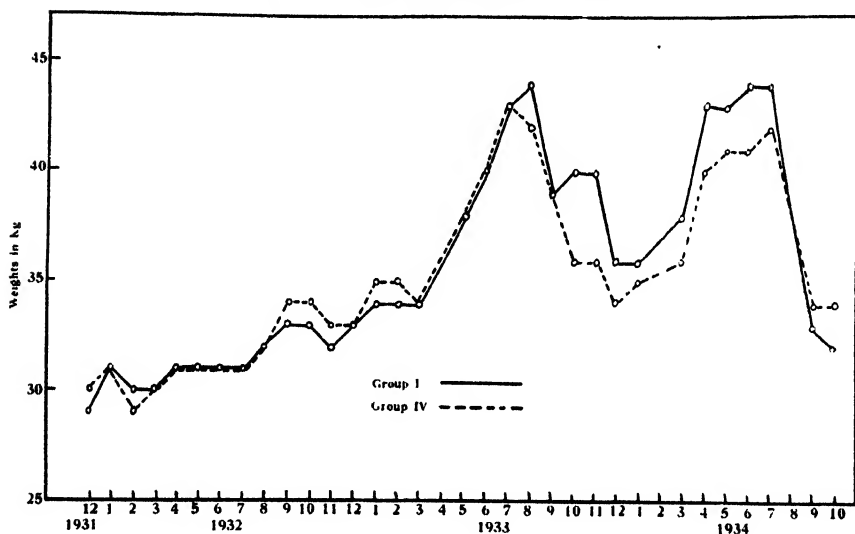


Fig. III. Monthly Weights of Sheep.

The animals were weighed in the morning after they had been kept away from water and hay for about 15 hours and this method was found to be very satisfactory for recording correct weights when weighing only once per month.

A glance at figures I, II and III reveals the fact that significant differences in the weight curves of the sheep are absent. Apparently therefore different quantities of KI were without effect on the weight increase of the sheep. The remarkably sudden drops in weight from August, 1933, and again from July, 1934, were due to lambing and subsequent lactation which will be dealt with more fully under reproduction. During these periods the decreases in weight were apparently not affected by the KI present.

*Wool Produced.*

A record of the wool produced in 1931, 1932 and 1934 respectively, each calculated on the basis of 365 days' growth, which can be done, as the annual growth of wool, according to Bosman (1935), takes place proportionately throughout the year, provided the conditions of feeding are not changed, is given in the table below:—

TABLE I.

Wool Production (grams) per 365 days.

(Clean weights expressed on dry basis and grease weights on airdry basis.)

*Group I.*

Experiment No.	Grease weight, 1931. Pre-experimental period.	Clean dry weight.	Grease weight, 1932. Experimental period 1st year.	Clean dry weight.	Grease weight, 1934. Experimental period 3rd year.
32882	3,878	1,556	3,474	1,347	ewe died
32889	4,119	1,692	3,713	1,538	3,438
32897	4,045	1,703	3,688	1,574	3,287
32898	3,612	1,608	3,550	1,547	3,080
32905	4,526	1,833	4,002	1,602	3,515
32886	3,617	1,499	3,435	1,365	3,421
32913	3,878	1,517	3,807	1,467	ewe died
32914	3,491	1,760	3,295	1,544	3,105
32921	3,954	1,784	3,081	1,441	ewe died
32890	4,097	1,858	ewe died	—	—
TOTAL.....	39,218	16,810	32,045	13,425	19,846
AVERAGE....	3,922	1,681	3,561	1,492	3,308

*Group II.*

Experiment No.	Grease weight, 1931. Pre-experimental period.	Clean dry weight.	Grease weight, 1932. Experimental period 1st year.	Clean dry weight.	Grease weight, 1934. Experimental period 3rd year.
32883	3,370	1,402	3,476	1,435	3,239
32888	4,242	1,822	3,702	1,500	3,459
32891	4,331	1,868	3,844	1,676	3,049
32896	3,587	1,560	3,239	1,443	2,877
32899	4,281	1,832	3,917	1,763	ewe died
32904	3,950	1,839	3,459	1,388	3,328
32907	3,342	1,420	3,467	1,514	3,207
32912	3,833	1,526	3,508	1,386	ewe died
32915	3,588	1,619	3,250	1,417	2,376
32920	3,503	1,431	3,313	1,316	ewe died
TOTAL.....	38,027	16,319	35,175	14,838	21,535
AVERAGE....	3,803	1,632	3,518	1,484	3,076

*Group III.*

Experiment No.	Grease weight, 1931. Pre-experimental period.	Clean dry weight.	Grease weight, 1932. Experimental period 1st year.	Clean dry weight.	Grease weight, 1934. Experimental period 3rd year.
32884	3,875	1,824	3,126	1,374	3,489
32887	3,778	1,909	3,052	1,532	2,998
32892	4,428	1,889	3,936	1,565	ewe died
32895	3,454	1,632	2,747	1,333	2,658
32900	3,583	1,503	3,417	1,476	3,475
32903	4,675	1,722	4,596	1,761	3,384
32908	3,400	1,465	3,358	1,187	3,381
32911	3,642	1,667	3,175	1,290	2,809
32916	4,035	1,708	3,444	1,428	2,851
32919	4,121	1,672	3,202	1,329	3,450
TOTAL.....	38,991	16,991	34,063	14,275	28,495
AVERAGE....	3,899	1,699	3,406	1,428	3,166

*Group IV.*

Experiment No.	Grease weight, 1931. Pre-experimental period.	Clean dry weight.	Grease weight, 1932. Experimental period 1st year.	Clean dry weight.	Grease weight, 1934. Experimental period 3rd year.
32885	4,409	1,893	3,886	1,700	3,839
32906	3,466	1,496	3,254	1,274	2,871
32893	4,104	1,691	3,539	1,535	3,347
32894	4,049	1,674	3,479	1,399	3,208
32901	4,398	1,794	3,618	1,356	3,798
32902	3,330	1,482	3,133	1,214	2,041
32909	3,745	1,674	3,196	1,325	3,081
32910	3,808	1,572	3,063	1,158	3,173
32917	3,501	1,472	2,711	1,140	2,514
32918	4,201	1,915	3,714	1,680	4,094
TOTAL.....	39,011	16,663	33,593	13,781	31,966
AVERAGE....	3,901	1,666	3,359	1,378	3,197

The 1931 and 1932 fleeces were scoured to obtain the weights of clean wool but as these results are entirely insignificant when the wool production of the different groups is compared for these two seasons, i.e. when no iodine was given (1931) and when iodine was given (1932) scouring the wool was discontinued and only the grease weights for 1934 are given. The amount of wool produced in 1933 was unfortunately not registered. A statistical analysis of the weights of wool produced revealed no significant difference. As a matter of fact the weights are remarkably constant. In the entire absence of significant differences in the weights of wool produced by the groups receiving KI when compared with the control group and with the wool weights prior to KI feeding it may reasonably be concluded that the quality of the wool was not affected by the feeding of KI to the sheep (Bosman 1935).

A summary of the wool production data appears in Table 2, which represents the wool production of the different groups during each of the four years that the sheep were under observation. This table gives the results of wool production at a glance.

TABLE II.  
*Average Wool Production of Groups (grams).*

	Supplements.	1931 clip.	1932 clip.	1934 clip.
Group 1.....	No iodide	3,922	3,561	3,308
Group 2.....	.002 gm. KI.	3,803	3,518	3,076
Group 3.....	.02 gm. KI.	3,899	3,406	3,166
Group 4.....	.06 gm. KI.	3,901	3,359	3,197

### *Reproduction.*

In the last publication (Malan *et al* 1932) it was concluded that .02 grm. KI had detrimental effects on reproduction and it was furthermore suggested that limiting factors such as phosphorus deficiency might intensify the effects of iodine feeding. In that experiment it appeared that the sheep on the lowest phosphorus intake and given KI aborted sooner and produced less live lambs than those on a higher P intake during the second lambing season. Even in the KI group receiving adequate phosphorus abortions occurred and lambing was a failure.

In considering the available data and the ill effects of KI the authors state the following: "Whether the detrimental effect was entirely due to the iodine administered is difficult to state positively, but that the iodide seemed to aggravate the state of affairs is almost a certainty, and apparently much more so in the group receiving a minimum of phosphorus in its ration." Subsequent work has revealed that vitamin A deficiency was an additional limiting factor and that, just as P deficiency appeared to exaggerate the effects of iodine feeding so vitamin A deficiency most probably acted similarly. The work on vitamin A deficiency which is in the course of being published reveals an interesting fact, viz. that in the experimental feeding of cattle and sheep, especially in experiments on mineral metabolism where purified foods are often given, provision has to be made for the adequate supply of vitamin A. If this is not done complications in reproduction may be anticipated.

On looking at the lambing charts of the present experiment in the light of the earlier work (1932) it must be remembered therefore that the results in the earlier work were complicated by a vitamin A deficiency which was eliminated from the latter work and which most probably intensified the results obtained in the earlier experiment.

With regard to the observations on oestrus, fuller details of which will be published at a later date, it should be mentioned that the iodine supplements were found to have no significant influence on the occurrence, periodicity or the duration of oestrus.

The lambing charts for 1933 and 1934 are given in tables III and IV below.

TABLE III.  
*Lambing Chart, 1933.*

No. of ewe and group.	Gestation period, days.	Details about lamb at birth.	Weight Kg.	Weight of lamb after 1 month.
<i>Group I.</i>				
32882	149	Normal lamb.....	2.7	Died of hunger 7 days after birth.
32889	148	" " .....	3.9	7.2 Kg.
32897	147	" " .....	4.4	9.4 "
32898	151	" " .....	4.2	7.9 "
32905	151	" " .....	3.4	Lamb died 3 days old. Ewe no milk.
32886	154	" " .....	3.6	10.2 Kg.
32913	147	" " .....	2.7	6.3 "
32914	151	" " .....	3.2	8.4 "
32921	158	Dead lamb partly decomposed		Lamb dead.
AVERAGES..	149.8	Average weight..	3.5	Average weight 8.2 Kg.
<i>Group II.</i>				
32883	155	Lamb horn dead...	4.6	Lamb dead.
32888	152	" " .....	4.0	" "
32891	150	Normal lamb.....	4.0	6.5 Kg.
32896	152	" " .....	4.7	9.3 "
32899	—	Ewe died pregnant	—	Ewe dead.
32904	147	Normal lamb.....	3.4	Died of hunger. Ewe not enough milk.
32907	150	" " .....	4.2	Died of Hunger. Ewe no milk.
32912	152	" " .....	3.4	5 Kg.
32915	150	" " .....	2.7	6.1 Kg.
32920	154	" " .....	3.7	Lamb died of hunger.
AVERAGES..	151.3	Average weight..	3.9	Average weight 6.7 Kg.
<i>Group III.</i>				
32884	153	Normal lamb.....	3.6	8.2 Kg.
32887	149	" " .....	3.7	6.7 "
32892	152	" " .....	4.6	10 "
32895	152	" " .....	3.8	Lamb died 3 days old, Ewe no milk.
32900	150	" " .....	3.7	Lamb died shortly after birth.
32903	152	" " .....	3.8	Lamb died " " "
32908	153	Born dead.....	4.9	" dead.
32911	149	Dystokia.....	4.8	Lamb dead, wrong preservation.
32916	151	Normal lamb.....	3.2	10 Kg.
32919	152	" " .....	3.5	5.9 "
AVERAGES..	151.3	Average weight..	4.1	Average weight 8.2 Kg.
<i>Group IV.</i>				
32885	150	Normal lamb.....	3.7	9.3 Kg.
32906	151	" " .....	3.8	6.2 "
32893	149	" " .....	4.0	9.1 "
32894	149	" " .....	4.5	8.8 "
32901	149	" " .....	3.0	7.9 "
32902	149	" " .....	4.7	10.2 "
32909	153	" " .....	4.7	8.4 "
32910	153	" " .....	4.6	8.6 "
32917	151	" " .....	3.5	8.1 "
32918	147	" " .....	4.1	7.9 "
AVERAGES..	150.1	Average weight..	4.1	Average weight 8.2 Kg.

TABLE IV.  
*Lambing Chart, 1934.*

No. of ewe and group.	Gestation period days.	Details about lamb at birth.	Weight, Kg.	Weight of lamb after 2 months.
<i>Group I.</i>				
32882	150	Dead lamb.....	3.0	Dead.
32889	153	Healthy lamb.....	2.9	6.8 Kg.
32897	154	" " .....	3.4	Died, age 3 days. Ewe no milk.
32898	145	" " .....	3.5	7.7 Kg.
32905	149	" " .....	2.3	10.4 "
32886	150	" " .....	2.7	Died, 3 days old. Ewe no milk.
32913	149	" " .....	3.0	Died, 2 days old. Ewe no milk.
32914	156	" " .....	3.4	10.4 Kg.
32921	ewe died	" —	—	—
AVERAGES..	150.8	Average weight..	3.2	Average weight 8.8 Kg.
<i>Group II.</i>				
32883	159	Dystokia.....	3.4	Died shortly after birth.
32888	152	Healthy.....	3.1	9.1 Kg.
32891	149	" .....	3.5	5.5 "
32896	160	" .....	3.3	Died, 21 days old. Ewe not enough milk.
32899	ewe died	—	—	—
32904	150	Healthy.....	3.3	10.4 Kg.
32907	149	" .....	3.5	Died, 12 days old. Dipping accident.
32912	148	" .....	3.6	Died of hunger, Ewe no milk.
32915	151	" .....	3.3	Died of hunger. Ewe not enough milk.
32920	148	Dystokia.....	3.9	Dead shortly after birth.
AVERAGES..	151.8	Average weight..	3.4	Average weight 8.3 Kg.
<i>Group III.</i>				
32884	No lamb	No lamb.....	—	No lamb.
32887	150	Healthy lamb.....	3.0	Died 2 days old. Ewe no milk.
32892	149	" " .....	4.1	Died. Ewe no milk.
32895	148	" " .....	4.0	10.9 Kg.
32900	150	" " .....	3.3	Died 24 days old. Ewe not enough milk.
32903	148	Dystokia.....	4.3	Died shortly after birth.
32908	148	Healthy lamb.....	3.9	6.8 Kg.
32911	147	Dead lamb.....	3.1	Dead.
32916	149	Healthy lamb.....	3.2	6.8 Kg.
32919	No lamb	No lamb.....	—	No lamb.
AVERAGES..	148.6	Average weight..	3.6	Average weight 8.2 Kg.
<i>Group IV.</i>				
32885	144	Healthy.....	3.7	Died 2 days old. Ewe no milk.
32906	150	" .....	3.6	7.7 Kg.
32893	150	" .....	3.8	6.4 "
32894	148	" .....	3.6	7.7 "
32901	135	" .....	2.9	10.4
32902	150	" .....	3.4	Died 19 days old. Ewe not enough milk.
32909	148	" .....	3.5	10.4 Kg.
32910	149	" .....	3.6	Died 1 day old. Ewe no milk.
32917	150	" .....	3.3	7.3 Kg.
32918	145	" .....	3.4	Died 2 days old. Ewe no milk.
AVERAGES..	146.9	Average weight..	3.5	Average weight 8.3 Kg.

Several interesting points are brought out in the lambing charts. In contrast to the lambing results reported in the earlier work where a vitamin A deficiency was present, no abortions took place.

It is noteworthy that Bekker (1931) also reported abortions subsequent to feeding KI to sheep. The dose (·076 gm.) given was slightly larger than that of Group IV (·06 gm. KI) and what appears to be more important, pregnancy and lambing occurred during a period of drought when the vitamin A content of the pasture was at its lowest. The probability of abortions after KI feeding during droughts has an important practical aspect and seems to justify further investigation.

Four full-time and overtime foetusses were born dead in July, 1933 and two in 1934 out of a total of 38 and 36 pregnant ewes respectively. The gestation periods during both seasons were apparently unaffected by the KI supplement. Neither the birth weights of the lambs nor their weights at the time of their discharge from the experiments showed any significant group differences. The number of lambs that died of hunger during both seasons on account of lack of milk is remarkable. This number appears to be significantly less in Group IV in July, 1933, but not so in July, 1934, and there is not enough evidence that any one group did better than the other in this respect. It was noticeable indeed that the ewes, while being in excellent condition at lambing lost weight very rapidly during lactation and that in a remarkable number of cases they had no milk or not enough to keep the lambs alive. A protein deficiency was suspected during this period and the matter is being further investigated. The number of deaths of lambs in the separate groups is, however, not significantly different and it can only be concluded that the KI supplement here too was without effect. Apparently therefore no visibly detrimental effects of iodine feeding was experienced in regard to reproduction, nor can it be said that the iodine acted beneficially.

After the removal of the lambs at the age of three months the ewes dried off and directly began gaining in weight without change of diet.

#### SUMMARY AND CONCLUSIONS.

1. Data are presented on the effect of KI in the feed of 40 merino ewes for a period of 30 months.
2. The quantities of KI given per sheep daily were ·002 gm., ·02 gm., ·06 gm. in three groups respectively, while one group received no KI and acted as controls.
3. Observations were made on the food consumption, weight increase, wool production, reproduction and health of the animals for the full period of the experiment. Further, observations were made on the oestrus cycle of the ewes and no significant group differences were observed.
4. The animals were fed in individual feeding boxes except the hay, which was given *ad lib.*



5. It is concluded that neither the small dose nor the bigger quantities of KI fed had any visible effect on the weights, wool production and reproduction of the animals.

6. It is also pointed out that the ill effects ascribed to iodide feeding in the earlier work were apparently aggravated by another deficiency, the possibility of the existence of which was not considered in the earlier work, viz. vitamin A.

7. It is concluded that the addition of KI to sheep licks is unwarranted, may even produce visably detrimental effects under conditions of drought, when a vitamin A shortage may exist, and is to be discouraged in practical farming unless there is reason for believing that an iodine deficiency exists.

8. Further work on the effects of iodine feeding under practical conditions, as for instance during severe droughts when apparently detrimental effects may be experienced, is being undertaken.

The writers wish to acknowledge gratefully the assistance of Mr. A. P. Malan, Statistician, in dealing with the results given in the lambing charts.

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# **A Study of the Mineral Content and Feeding Value of Natural Pastures in the Union of South Africa.**

## **THIRD REPORT.**

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## **INTRODUCTION AND PLAN OF WORK.**

In the first publication (du Toit *et al.*, 1932) of the above series the plan of investigation was fully described, the objects, methods employed and technique were given in detail. The results of the first three surveys were recorded and discussed. A later publication (du Toit *et al.*, 1933) reported on the results obtained in the plot experiments which formed a sub-division of the original experimental plan. The analyses of six more surveys, i.e. until April, 1933, have now been completed and are reported on in this article.

Briefly, the surveys meant collecting samples of pasture at regular intervals from a number of areas all over the Union and analysing these for crude protein, crude fibre, soluble extract, soluble ash, phosphorus, calcium, magnesium, sodium, potassium and chlorine. Samples of soil and blood were collected simultaneously from the same area or from animals grazing in that area, the former analysed for inorganic constituents and nitrogen and the latter for phosphorus. In the first publication mentioned above the tables of results included values for both soil and pasture constituents and for blood phosphorus. The view was expressed, however, that soil analysis does not provide a very satisfactory method of studying the feeding value of pastures as obviously pasture on poor soil may yield excellent values if the samples for analysis are taken at an early stage of growth of the pasture. Hence, soil analyses, which entailed a considerable amount of labour have been omitted from further work and the number of areas from which pasture samples have been collected increased accordingly.

The diagnosis of phosphorus deficiency in pasture by determining the inorganic phosphorus content of the blood of animals grazing such pasture is simple and accurate, but the surveys described in this series of articles were intended to be a study of the feeding value of South African pastures and not merely a study of their phosphorus content. Besides, from the large number of figures for blood phosphorus it has already become evident that low phosphorus in the blood, and therefore in the pasture, is the rule rather than the exception, so that pasture analysis, which is essential for a study of the greater problem of the feeding value of the pastures and which incidentally includes the determination of phosphorus in the pasture, already covers the field of phosphorus deficiency. Blood analysis has therefore served the purpose of establishing in a remarkably short period the fact that practically all South African pastures are deficient in phosphorus at certain seasons of the year. Blood analysis for phosphorus is now being used for studying the phosphorus deficiency problem in herds or on farms where immediate information on this problem is required, and has been excluded from the greater surveys, where it would naturally involve the bleeding of many hundreds of stock over extended periods, a procedure which after several repetitions is generally met with a certain amount of opposition on the part of the farmers. Another obstacle in the way of drawing the blood and preparing it for despatch to the laboratory for analysis is that a large number of areas have to be included in the surveys in order to obtain representative figures and the staff is not available to do the blood work in such a large number of areas simultaneously. On the other hand, Government Stock Inspectors are stationed practically all over the Union, and these men collect monthly samples of pasture on certain farms in their areas as part of their official duties. The organization for obtaining the pasture samples is therefore very simple and efficient. The number of areas from which pasture samples are collected has been greatly increased recently by employing the stock inspectors for this work rather than the field veterinary officers, whose numbers are limited. However, all the surveys reported on in this paper have been collected on the old basis described in the first article with one or two minor modifications as indicated below.

Soil analyses, as already explained, have been eliminated. The samples of vegetation are no longer sorted into species on arrival and these identified, but they are freed from sand, reduced if necessary, milled and analysed. The obvious disadvantage of this procedure is that figures are obtained which are not truly representative of the pasture actually eaten but of all the pasture in a particular area.

The above disadvantage has been overcome in all the recent work, about which later particulars will appear in due course, by actually following grazing animals in the veld and collecting samples of pasture while observing the animals' method of grazing, selecting of grasses, etc. In other words, the sample collected is as nearly as possible identical with the pasture eaten by the animals which were being followed.

For the surveys reported on in this article samples of pasture were received at all stages of growth so that there was no advantage in comparing the analysis of a particular species of grass from a

certain area with the values obtained for the same species from another area. The results are in any case not comparable, for, apart from other climatic and soil influences, the difference in stage of growth of the two samples of the same species from different areas could readily account for the difference in composition. The separate analysis of individual species of vegetation was therefore omitted from the scheme. The description of the samples was continued as before and the analyses carried out as described in the last publication (1934).

The method for determining soluble ash is given by Louw (1934). The other constituents were determined according to the methods given in the earlier publications.

Briefly, then, the investigation included the collection of pasture samples simultaneously from a number of areas at three-monthly intervals and the period to be reported on this publication ranged from January, 1932, until April, 1933. The samples were described on reaching the central laboratory at Onderstepoort, dried and milled. The following determinations were then made and calculated on absolute dry basis: Soluble ash (omitted from the present series), crude protein, crude fibre, phosphorus, calcium, magnesium, potassium, sodium, chlorine and nitrogen free extractives plus ether soluble extract. The omission of one or two important determinations from the present series such as soluble ash was governed by available assistance and as large numbers of samples were arriving it was necessary to remain up to date with the work in order to keep the organization of collecting, forwarding, registering and preparing the samples for analysis active and efficient even at the sacrifice of determinations which could have been included with advantage. However, such inadvertent omissions in the earlier work have been rectified, the organization of both field and laboratory staff perfected as the officers, especially in the field, became better acquainted with the work with the result that at present an organization has developed for the collection of several hundreds of samples monthly by stock inspectors stationed in as many areas in the Union, for the despatch of these samples to Onderstepoort, where the registration, preparation for analyses and actual determinations are carried out efficiently and in such a way that the farmers and officers in the areas concerned are informed from time to time of the results of the investigation and the fruits of their labour and co-operation.

## RESULTS.

Table I gives the monthly rainfall in the areas of collection for the period November, 1931, April, 1933.

The values given in Table I, not being registered actually at the sites of the collection of the pasture samples, should be taken to be approximate and not absolute. The rainfall table will be discussed in conjunction with the values obtained on analysis of the pasture samples which are given in Table II.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE I.  
Rainfall in Inches.

Farm and District.	Nov. 1931.	Dec. 1931.	Jan. 1932.	Feb. 1932.	Mar. 1932.	April 1932.	May 1932.	June 1932.	July 1932.	Aug. 1932.	Sept. 1932.	Oct. 1932.	Nov. 1932.	Dec. 1932.	Jan. 1933.	Feb. 1933.	Mar. 1933.	April 1933.
Derby No. 59, Piet Retief.....	6.38	4.69	4.50	7.09	5.69	1.01	2.85	0.84	nil	nil	1.34	2.72	3.87	7.68	2.08	—	—	—
Wildbeestfontein, Pletasburg.....	2.88	3.80	3.35	1.46	3.38	2.23	0.75	nil	nil	nil	nil	0.75	2.75	2.65	8.07	—	—	—
Mimosa Park, Potchefstroom.....	3.25	3.64	2.35	1.46	3.38	2.23	0.75	nil	nil	nil	1.08	0.95	1.09	4.20	—	—	—	—
Stainton, Kropp.....	3.27	3.35	3.35	4.79	3.25	0.19	3.25	1.20	0.50	0.50	1.33	3.01	4.91	5.36	3.11	2.56	4.39	1.35
Bengand, Vryheid.....	1.35	2.50	2.24	6.01	4.00	0.59	3.26	0.16	nil	nil	0.15	0.86	3.37	6.00	1.58	3.06	0.64	0.96
Lech Sloy, Kestour.....	—	—	4.93	6.18	3.31	2.11	2.30	0.16	nil	nil	1.63	0.93	4.65	5.19	1.57	Not	forwarded.	—
Zulu Native Training Institute.																		
Nongoma.....	2.90	2.21	1.79	2.73	3.13	4.75	4.25	0.39	nil	nil	1.06	1.72	3.18	4.97	4.01	2.55	3.65	—
Moss Side, Dundee.....	2.17	2.87	3.69	4.99	5.41	0.22	2.39	0.28	nil	nil	0.86	0.93	2.79	4.99	—	—	—	—
Mariannhill, Pinetown.....	4.36	3.01	6.10	5.10	4.20	1.00	3.42	nil	0.12	0.03	1.14	4.87	4.04	4.85	2.05	2.37	2.74	1.03
Commissie Drift, Richmond.....	3.60	5.23	4.66	4.41	4.96	0.75	1.95	nil	0.34	0.16	1.39	3.27	3.94	5.81	2.39	2.92	4.05	1.33
Lots 45/43, Entumeni, Eschewe.....	3.53	5.91	5.45	17.26	7.03	7.11	6.50	0.78	0.10	1.89	1.60	5.06	4.24	8.46	7.09	6.50	3.51	—
Voorkeur, Umvoti.....	—	—	4.39	11.57	6.63	0.62	3.01	0.24	0.08	nil	1.73	2.94	4.91	6.01	5.86	3.77	3.99	1.52
Melbourne, Port Shepstone.....	1.37	2.86	6.30	8.09	2.44	0.25	0.86	0.60	0.67	0.85	3.99	4.59	6.99	6.37	2.30	—	—	—
Rockdale, Kingwillamstown.....	2.37	4.18	2.60	5.97	2.10	nil	1.67	0.12	1.65	0.35	5.29	2.43	6.54	2.76	—	—	—	—
Koppieskraal, Mt. Currie.....	2.44	3.53	2.69	6.02	3.44	0.18	1.72	1.07	1.09	0.09	2.20	2.46	6.86	3.02	1.75	1.40	4.59	—
Xura Tank Area, Lusitlaiki.....	3.57	4.57	2.24	8.71	3.09	0.16	3.40	1.43	2.03	0.27	6.43	3.76	11.94	5.74	0.72	—	—	—
Craddock Place, Port Elizabeth.....	—	—	4.32	2.13	6.37	0.12	1.27	1.12	4.39	1.50	13.43	4.40	4.25	0.49	2.61	3.34	2.10	2.18
Princeton, Bedford.....	0.73	0.12	1.87	1.02	1.03	nil	2.11	0.47	1.95	nil	5.22	2.47	3.88	—	—	Not given.	—	—
Lombardspoot, Bathurst.....	0.69	6.15	4.35	2.20	1.26	0.05	1.40	0.69	1.99	0.68	3.89	3.05	3.72	1.14	0.50	0.93	0.93	—
Woodvale, Albany.....	0.92	1.56	2.95	1.53	0.46	0.24	1.19	0.13	0.78	nil	3.89	2.35	0.94	1.06	0.83	0.36	1.39	1.19
Alhendale, Middelburg, Cape.....	5.08	0.76	0.43	3.38	2.86	nil	0.35	0.08	0.49	nil	—	0.45	—	—	1.00	1.00	1.00	0.50
Naseby Thorns, Kroonstad.....	4.85	1.32	1.20	5.01	3.10	0.27	nil	nil	nil	nil	nil	nil	0.76	—	0.86	1.12	1.79	2.20
The Outlook, Bethlehem.....	1.92	1.17	1.76	4.06	2.12	0.17	nil	nil	1.33	0.10	3.76	3.29	5.64	1.60	1.41	2.10	4.67	—
Mimosa Farm, Umata.....	1.37	3.61	3.08	3.83	0.56	0.18	0.97	0.26	—	0.83	1.93	4.60	3.73	2.68	1.33	1.91	1.05	1.94
Roodepoort, Middelburg, Tvl.....	2.52	3.29	2.87	3.93	3.24	0.28	0.62	nil	—	0.45	0.49	1.60	2.93	3.51	3.75	1.28	6.02	0.99
Brooklyn, Barberton.....	2.47	2.68	3.41	3.03	3.80	1.03	nil	nil	—	0.02	0.34	nil	4.12	5.20	—	—	—	—
Gov. Ranching Sta., Zoutpansberg.....	2.94	1.45	2.56	4.21	5.77	1.47	0.06	nil	—	0.05	0.04	1.10	0.93	5.02	5.13	Not given.	—	—
Georgiats, Marico.....	2.70	2.68	3.41	3.03	3.80	1.63	1.14	nil	—	nil	1.26	0.54	—	—	—	—	—	—
Georgiats, Mankoring.....	2.54	6.00	0.53	1.99	1.67	2.38	nil	—	—	nil	0.92	0.92	—	—	—	—	—	—
Offiantveld, Mankoring.....	2.46	3.43	1.94	2.55	3.13	2.35	0.11	—	—	nil	0.89	0.54	—	—	—	—	—	—
Melrose Farm, Zoutpansberg.....	2.46	3.43	1.94	2.55	3.13	2.35	0.11	—	—	0.15	1.80	0.89	1.20	0.52	—	—	—	—
Mt. Humpy, Zoutpansberg.....	1.23	2.80	1.06	3.17	0.15	0.33	0.20	0.86	—	0.15	1.80	0.89	1.20	0.52	—	—	—	—
Leifontein, Ermelo.....	6.23	2.03	—	—	—	1.09	1.07	1.46	—	—	1.23	1.62	to have been	3.99	5.19	2.28	1.57	—
Rustfontein, De Aar.....	2.82	3.00	10.90	2.45	1.98	0.70	2.46	1.46	—	—	1.30	0.88	1.12	1.64	1.28	1.97	0.40	0.62
Homesfarm, Klip River, Natal.....	2.68	0.78	5.60	3.38	0.67	0.33	0.11	0.20	—	—	1.08	0.68	1.12	1.64	1.28	1.97	0.40	0.62
Churchill, Kuruman.....	2.24	3.69	1.89	3.99	0.85	0.05	0.92	1.03	—	—	3.51	1.80	6.61	1.94	0.92	1.91	3.02	3.39
The Gaol, Butterworth.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE II.  
*Surveys IV, V, VI, VII, VIII and IX were carried out during January, 1932, April, 1932, July, 1932, October, 1932, January, 1933, and April, 1933.*

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	Cl	Crude Protein.	Crude Fibre	Nature of Pasture	Average Mgm. % F.	Animals bled.
<i>Transvaal Province</i>												
Moolwater No. 129, Middelburg, Tvl.	IV	12	28	11	36	01	08	4 1	34 6	Mixed, mainly green.	5 4	Dry cows.
Rodepoort No. 8, Middelburg, Tvl.	V	15	31	18	83	01	19	4 5	36 1	Mixed, mainly green.	3 4	Lactating cows.
Besbokvlakte No. 8, Middelburg, Tvl.	VI	08	29	18	49	Trace	10	2 3	35 0	Brown.	3 7	Dry cows.
Walden No. 409, Middelburg, Tvl.	VII	14	38	20	70	Trace	16	3 9	34 5	Mixed, mainly leaves.	2 3	Working oxen.
Walden No. 128, Middelburg, Tvl.	VIII	15	31	17	29	02	29	5 0	35 5	Mixed.	2 8	Working oxen.
Walden No. 128, Middelburg, Tvl.	IX	11	43	19	29	01	17	4 4	35 9	Mixed.	2 3	Dry cows.
Hartebeestfontein No. 51, Krugersdorp	IV	16	24	17	60	02	14	5 1	34 1	Mixed, mainly green.	4 0	Oxen.
" " " " " " " "	V	12	19	16	68	01	14	3 5	38 1	Mixed, mainly brown.	4 2	Oxen.
" " " " " " " "	VII	12	24	24	77	Trace	12	5 2	38 9	Mixed, mainly brown.	4 0	Oxen.
Zuurbekom No. 9, Krugersdorp	VIII	13	14	18	62	01	16	5 7	38 8	Mixed, mainly green.	3 2	Oxen.
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Brooklyn, Barberton	IV	21	39	20	65	02	16	4 2	41 5	Mixed.	6 5	Helpers.
" " " " " " " "	V	19	34	19	47	02	08	3 0	44 5	Mixed, mainly brown.	5 2	Helpers and oxen.
Castlekop, Barberton	VII	47	27	24	2 08	04	43	4 3	40 4	Mixed, mainly green.	4 4	Helpers and oxen.
" " " " " " " "	VIII	37	30	22	1 60	02	29	2 6	43 6	Mixed.	5 1	Dry cows and oxen.
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Kaaplaats, Marico	IV	09	25	11	37	01	09	4 5	36 5	Mixed.	—	—
" " " " " " " "	V	05	26	15	42	Trace	09	2 3	41 6	Mixed.	—	—
" " " " " " " "	VI	08	22	13	41	01	09	3 2	41 0	Brown.	3 1	Dry ewes.
Bietgat No. 197, Marico	VII	08	26	18	41	01	09	3 2	41 0	Brown.	4 8	Dry ewes.
" " " " " " " "	VIII	23	23	24	1 12	05	30	6 0	39 6	Mixed, mainly green.	5 2	Dry ewes.
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Gemsbokpan, Mafeking	IV	10	25	11	78	01	12	3 2	34 1	Mixed.	—	—
" " " " " " " "	V	13	33	15	78	01	11	5 8	37 0	Mixed, mainly green.	—	—
" " " " " " " "	VI	08	27	15	56	01	10	3 7	38 9	Brown.	2 4	Dry ewes and lactating cows.
Welgemoed Wes, Mafeking	VII	09	22	12	41	02	07	3 7	37 6	Brown.	3 0	Dry ewes and lactating cows.
" " " " " " " "	VIII	15	35	21	1 63	02	31	6 5	37 5	Mixed.	3 2	Dry ewes.
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Ollantsoetsi, Marico	IV	24	53	24	1 60	03	45	5 8	34 4	Mixed.	3 5	Cows in milk.
" " " " " " " "	V	20	30	26	1 56	01	32	4 7	37 2	Mixed, mainly brown.	3 8	Lactating cows.
" " " " " " " "	VI	14	33	16	79	02	17	2 5	39 9	Brown.	2 5	Lactating cows.
" " " " " " " "	VII	11	33	16	35	01	03	2 9	40 3	Brown.	4 3	Dry cows.
" " " " " " " "	VIII	—	—	—	—	—	—	—	—	—	—	—
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Church Hill, Kuruman	IV	18	57	24	1 03	02	27	4 8	34 8	Mixed, mainly green.	—	—
" " " " " " " "	V	05	34	11	37	Trace	08	2 0	37 4	Mixed, mainly brown.	—	—
" " " " " " " "	VI	09	45	22	63	02	08	2 0	40 1	Mixed, mainly brown.	—	—
" " " " " " " "	VII	08	40	22	63	01	08	2 1	37 6	Mixed, mainly brown.	—	—
" " " " " " " "	VIII	10	81	20	50	01	14	4 3	36 6	Mixed, mainly brown.	—	—
" " " " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—

TABLE II (continued).

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub>	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Crude Fibre.	Nature of Pasture.	Average Mgm. % F.	Animals bled.
Melrose Farm, Zwartkopsburg.	IV	.34	.34	.27	1.58	.02	.32	3.6	33.4	Mixed, seeds present..	6.7	Dry ewes.
" " " "	V	.15	.33	.27	.78	.02	.16	3.8	34.1	Mixed, .....	3.9	Pregnant ewes.
" " " "	VII	.13	.40	.33	.71	.01	.18	2.3	36.4	Mixed, mainly brown.	2.9	Dry and lactating ewes.
" " " "	VIII	.09	.31	.21	.40	.02	.09	2.2	38.5	Mixed, mainly brown.	5.9	Lactating ewes.
" " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Gov. Ranching Stn., Zoutpansburg	IV	.43	.63	.36	1.98	.18	.21	5.9	40.9	Mixed, mainly green..	5.4	Dry cows.
" " " "	V	.27	.39	.24	.94	.05	.15	3.7	40.9	Mixed, mainly green..	4.0	Dry cows.
" " " "	VII	.27	.46	.23	.66	.13	.17	3.0	41.9	Brown, .....	4.4	Dry cows.
" " " "	VIII	—	—	—	—	—	—	—	—	—	—	Dry and lactating cows.
" " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Mooiogelegen No. 635, Potgietersrust	IV	—	—	—	—	—	—	—	—	—	3.1	Lactating cows.
" " " "	V	—	—	—	—	—	—	—	—	—	4.3	Dry cows.
" " " "	VI	—	—	—	—	—	—	—	—	—	3.7	Dry cows and heifers.
" " " "	VII	—	—	—	—	—	—	—	—	—	3.9	Lactating cows.
" " " "	VIII	—	—	—	—	—	—	—	—	—	3.9	Lactating cows.
" " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Derby No. 56, Piet Retief.	IV	.13	.40	.14	.76	.05	.26	4.2	38.5	Mixed, .....	4.5	Dry cows.
" " " "	V	.11	.35	.15	.03	.03	.28	3.2	43.7	Mixed, mainly brown..	3.1	Dry cows.
" " " "	VI	.08	.44	.21	.38	.05	.12	2.2	39.0	Brown, .....	6.3	Dry cows.
" " " "	VII	.19	.34	.21	.54	.02	.32	5.1	37.2	Mixed, mainly green..	4.1	Dry cows and heifers.
" " " "	VIII	.15	.23	.14	.80	.03	.29	5.1	38.5	Mixed, .....	3.8	Dry cows and heifers.
" " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Wildebeestfontein, Pietersburg.	IV	.18	.33	.18	.56	.06	.18	3.4	36.0	Mixed, .....	3.9	Dry cows.
" " " "	V	.19	.37	.19	.54	Trace	.11	2.5	38.2	Mixed, mainly brown.	5.1	Dry cows.
" " " "	VII	.18	.33	.12	.31	.02	.07	2.8	37.8	Mixed, mainly brown.	4.1	Dry cows.
" " " "	VIII	.34	.36	.24	2.00	.03	.32	8.8	32.5	Mixed, mainly green..	3.1	Dry cows.
" " " "	IX	—	—	—	—	—	—	—	—	—	3.9	Dry cows.
Mimosa Park, Potchefstroom.	IV	.13	.28	.12	.72	.02	.15	5.1	35.7	Mixed, mainly green..	3.6	Dry and lactating cows.
" " " "	V	.12	.32	.21	1.01	.01	.22	4.0	35.5	Mixed, .....	3.4	Dry and lactating cows.
" " " "	VI	.09	.30	.17	.46	Trace	.09	2.7	38.3	Mixed, mainly brown..	2.3	Dry cows.
" " " "	VII	.10	.25	.12	.26	.01	.06	2.6	39.0	Mixed, mainly brown..	3.8	Dry cows.
" " " "	VIII	.20	.33	.22	1.43	.02	.32	6.0	36.4	Mixed, mainly green..	3.2	Dry and lactating cows.
" " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Leliefontein, Ermelo.	IV	.17	.22	.15	.91	.02	.20	4.4	33.8	Mixed, mainly green..	2.4	Lactating cows.
" " " "	V	—	—	—	—	—	—	—	—	—	—	—
Leliefontein, Ermelo.	VI	.13	.20	.14	1.13	.01	.11	3.3	36.4	Brown, long, .....	5.3	Lactating cows.
" " " "	VII	.22	.26	.16	.92	.04	.20	4.1	37.9	Mixed, mainly brown..	—	—
Kranspan No. 95, Ermelo.	VIII	.17	.25	.19	1.00	.01	.23	6.1	31.8	Mixed, mainly green..	3.8	Oxen.
" " " "	IX	.12	.29	.19	.88	.01	.23	4.8	33.9	Brown, .....	4.8	Oxen and dry cows.

TABLE II (continued).

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub> .	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Crude Fibre.	Nature of Pasture.	Average Mgm. % P.	Animals bled.
<i>Natal Province.</i>												
Stanton, Ixopo....	IV	.18	.21	.18	.92	.19	.30	5.8	40.9	Mixed, mainly green..	5.5	Dry cows.
" " " " " "	V	.19	.22	.32	1.34	.02	.16	4.3	41.9	Mixed, mainly green..	4.4	Young heifers.
" " " " " "	VI	.07	.27	.22	.35	.08	.17	2.3	41.5	Mixed, mainly brown.	4.1	Oxen.
" " " " " "	VII	.15	.17	.15	1.05	.10	.32	7.3	41.5	Green, mostly .....	4.0	"
" " " " " "	VIII	.15	.19	.17	1.06	.08	.42	5.0	43.6	Green, mainly green..	4.0	Dry cows and heifers.
" " " " " "	IX	.10	.23	.17	1.52	.02	.18	3.6	37.9	Mixed, mainly brown.	3.1	Heifers in calf.
Bergendal, Vryheid....	IV	—	—	—	—	—	—	—	—	—	—	—
" " " " " "	V	.15	.38	.34	1.05	.02	.30	6.2	37.5	Green, long.....	3.3	Lactating cows.
" " " " " "	VI	—	—	—	—	—	—	—	—	—	—	—
" " " " " "	VII	.27	.40	.25	.83	.01	.22	5.2	41.0	Mixed, short.....	3.9	Dry cows.
" " " " " "	VIII	.13	.44	.20	.58	.01	.23	3.3	36.0	Mixed.....	4.3	Merino wethers.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Kimbalton, Estcourt....	IV	—	.31	.18	.89	.02	.16	3.7	38.5	—	—	—
Loch Sloy, Estcourt....	V	.24	.50	.26	.94	.02	.31	3.1	36.0	Mixed, mainly brown.	4.4	Dry cows and oxen.
" " " " " "	VI	—	—	—	—	—	—	—	—	—	—	—
" " " " " "	VII	.22	.30	.17	1.24	.03	.23	5.3	39.9	Mixed.....	4.7	Young steers.
" " " " " "	VIII	.15	.40	.19	.80	.01	.17	4.4	37.6	Mixed.....	3.1	Cows in calf.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Zulu Nat. Training Inst., Nongoma.	IV	.36	.67	.50	2.30	.96	.88	11.5	32.8	Mixed, mainly green..	—	—
" " " " " "	V	.31	.42	.52	1.83	.28	.78	6.7	39.4	Mixed, mainly green..	7.9	Dry cows and oxen.
" " " " " "	VI	.20	.32	.31	.81	.11	.45	4.2	36.2	Mixed.....	5.0	Oxen.
" " " " " "	VII	.19	.34	.31	.64	.09	.26	4.1	39.8	Mixed, mainly brown.	6.0	Oxen.
Tokazi Farm, Nongoma..	VIII	—	—	—	—	—	—	—	—	—	—	—
" " " " " "	IX	.29	.32	.28	1.03	.22	.63	5.2	39.5	Mixed.....	6.4	Working oxen.
Moss Side, Dundee....	IV	.13	.28	.13	.84	.03	.22	3.7	40.6	Mixed.....	3.2	Dry cows and oxen.
" " " " " "	V	.19	.23	.18	1.30	.05	.27	3.3	41.7	Mixed, mainly green..	4.1	Dry cows and oxen.
" " " " " "	VI	.07	.25	.15	.47	Trace	.11	1.8	44.3	Mixed, mainly brown.	2.6	Dry cows and oxen.
" " " " " "	VII	—	—	—	—	—	—	—	—	—	—	—
Moss Side, Dundee....	VIII	.17	.20	.13	1.30	.02	.21	4.1	40.1	Mixed, mainly brown.	—	Working oxen and dry cows.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Marianhill, Pinetown....	IV	.17	.19	.14	.48	.05	.16	4.3	39.7	Green, seeds present.	5.5	Oxen.
" " " " " "	V	.11	.17	.07	.26	.04	.08	3.1	42.5	Mixed, long.....	4.8	Oxen.
" " " " " "	VI	.06	.20	.08	.24	.01	.09	2.2	43.6	Mixed, mainly brown.	4.1	Oxen.
" " " " " "	VII	.11	.18	.08	.26	.03	.07	3.8	42.0	Mixed.....	—	—
" " " " " "	VIII	.15	.22	.08	.28	.03	.16	5.0	42.5	Mixed.....	4.4	Working oxen.
" " " " " "	IX	.17	.17	.09	.69	.03	.20	5.9	42.1	Mixed.....	4.5	Oxen.



## MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE II (continued).

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub> .	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Crude Fibre.	Nature of Pasture.	Average Mgm. % P.	Animals bled.
Home Farm, Klip River, Natal.....	IV V VI VII VIII IX	.37 — .08 .12 —	.52 — .64 .48 —	.24 — .24 .17 —	1.91 — .70 .35 —	.03 — .01 .02 —	.28 — .15 .07 —	6.5 — 2.5 3.8 —	40.8 — 35.1 36.4 —	Mixed, mainly green.. — Brown.. Mixed, mainly brown.. —	— — 3.9 3.1 2.9 —	— Pregnant heifers. Pregnant heifers. Pregnant heifers.
Braeburn, Pietermaritzburg.....	IV V VI VII VIII IX	.24 .26 .28 .31 .51 .33	.26 .25 .28 .21 .22 .22	.15 .18 .22 .14 .14 .21	.79 — .84 .50 .02 .94 1.43	.06 — .04 .04 .03 .22 .02	.22 — .23 .13 .21 .55 .51	4.2 — 3.8 4.4 8.1 6.9 3.2	37.2 — 39.3 39.3 38.2 38.2 43.4	Mixed.. Mixed.. Mixed, mainly brown.. Mixed.. Mixed, mainly green.. Mixed.. —	5.0 — 6.2 6.3 6.0 5.9 5.3	Working oxen. Oxen. Oxen. Oxen. Young oxen. Young oxen.
Lots 45/48, Entumeni, Eshowe.....	IV V VI VII VIII IX	.23 .18 .15 .21 .19 .30	.26 .26 .21 .23 .26 .22	.18 .23 .15 .15 .20 .13	1.48 1.25 — .57 .38 1.01	.37 — .21 .08 .31 .04	.57 — .49 .32 .74 35	4.6 — 2.8 3.1 4.1 3.7	34.6 — 43.9 38.0 44.6 45.6	Mixed, mainly green.. — Mixed, long.. Mixed, mainly brown.. Mixed.. Mixed.. Mixed, mainly green.. —	7.2 — 5.0 3.4 4.3 4.3 4.5	Young heifers. Heifers and dry cows. Heifers and dry cows. Heifers and dry cows. Dry cows. Dry cows.
Voorkeur, Umvoti.....	IV V VI VII VIII IX	.18 .09 .23 .19 .11	.39 — .29 .34 .30 .24	.22 — .13 .20 .17 .11	1.01 — .48 1.19 1.40 .57	.03 — .01 .06 .03 .01	.28 — .16 .38 .37 .18	4.2 — 3.2 8.1 3.9 3.9	38.2 — 36.7 37.7 38.2 41.0	Mixed, mainly green.. — Mixed, mainly brown.. Mixed, short.. Mixed, mainly green.. Mixed, mainly green.. —	4.1 — 4.6 3.9 4.2 4.4 6.6	Lactating and dry cows. — Dry cows. Dry cows. Dry cows. Dry cows.
Melbourne, Port Shepstone.....	IV V VI VII VIII IX	.11 .10 .06 .17 .10 —	.22 .33 .25 .25 .23 —	.11 .22 .18 .14 .12 —	.60 — .66 .52 .52 .47	.11 — .11 .12 .19 .06	.34 — .25 .34 .34 .16	3.4 — 3.5 3.0 3.1 3.2	33.9 — 38.5 36.4 38.4 39.8	Mixed, short.. — Mixed.. Mixed, mainly brown.. Mixed, mainly brown.. Mixed, mainly brown.. —	8.7 — 8.1 2.7 2.4 2.7	Lactating cows. — Lactating cows. Lactating cows. Lactating cows. Lactating cows.

TABLE II (continued).

Farm and District.	Survey.	P.O. <sub>4</sub>	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Nature of Pasture.	Average Ngn. % F.	Animals bred.
<i>Cape Province.</i>											
Woodlands, Butterworth.....	IV	.21	.27	.12	.89	.12	.41	36.9	Mixed, mainly green..	—	—
" " " " " "	V	.09	.24	.12	.47	.05	.25	38.6	Mixed, mainly brown..	—	—
" " " " " "	VI	.08	.24	.14	.33	.06	.17	35.1	Mixed, mainly brown..	—	—
" " " " " "	VII	.13	.25	.12	.40	.07	.14	40.2	Mixed, mainly brown..	—	—
" " " " " "	VIII	.18	.26	.17	.88	.10	.41	36.6	Mixed, mainly brown..	—	—
" " " " " "	IX	.19	.37	.15	.89	.08	.28	36.4	Mixed.....	—	—
Teko School of Agric., Butterworth	IV	.13	.25	.14	.77	.18	.48	37.3	Mixed, short.....	—	—
" " " " " "	V	.08	.26	.13	.54	.10	.27	38.7	Brown.....	—	—
" " " " " "	VI	.10	.30	.16	.80	.06	.14	39.9	Mixed, mainly brown..	—	—
" " " " " "	VII	.13	.32	.13	.82	.04	.36	38.2	Mixed, mainly brown..	—	—
" " " " " "	IX	.20	.36	.16	.70	.07	.26	35.3	Mixed.....	—	—
Mt. Hupeley, Queenstown.....	IV	.20	.33	.13	.95	.03	.19	35.3	Mixed, mainly green..	3.2	Cows in milk.
" " " " " "	V	.14	.32	.13	.88	.02	.16	34.0	Mixed, mainly brown..	5.0	Young heifers.
" " " " " "	VI	.09	.23	.16	.37	Trace	.06	33.9	Mixed, mainly brown..	5.2	Heifers and oxen.
Combeleigh, Queenstown.....	VII	.21	.35	.20	.76	.02	.18	34.5	Mixed, mainly green..	4.9	Heifers and dry cows.
" " " " " "	VIII	.15	.30	.17	.71	.01	.53	36.5	Mixed, long.....	5.7	(Cows in milk (add. feed).)
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—
Rustfontein, De Aar.....	IV	.69	.57	.28	2.83	.03	.33	10.6	(Cynodon dactylon only) Mixed, mainly green..	5.3	Heifers, oxen and dry cows.
" " " " " "	V	.38	.44	.27	.87	.02	.13	4.9	Mixed, mainly green..	4.0	Heifers, oxen and dry cows.
" " " " " "	VI	.29	.37	.25	.52	Trace	.07	4.6	Mixed, mainly brown..	3.0	Heifers, oxen and dry cows.
" " " " " "	VII	—	—	—	—	—	—	—	—	—	—
" " " " " "	VIII	Could not collect	—	—	—	on account of severe drought.	—	—	—	—	—
" " " " " "	IX	.54	.87	.41	2.09	.04	.78	41.5	Mixed, and bushes....	—	—
Rockdale, Kingwilliamstown ..	IV	.25	.28	.14	1.01	.08	.33	5.8	Mixed, mainly green..	3.5	Dry and lactating cows.
" " " " " "	V	.16	.24	.18	.95	.08	.34	5.0	Mixed.....	3.6	Dry and lactating cows.
" " " " " "	VI	.36	.36	.16	.66	.05	.15	5.0	Mixed, mainly brown..	4.2	Dry cows and heifers.
" " " " " "	VII	.64	.42	.22	1.74	.29	.52	14.0	Green, short.....	4.5	Dry pregnant cows and heifers.
" " " " " "	VIII	.61	.38	.21	1.90	.17	.46	8.8	Green.....	4.2	Dry cows and heifers.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—
Kopjeskraal, Mt. Currie.....	IV	.27	.34	.16	1.03	.04	.25	5.4	Mixed, mainly green..	5.6	Heifers, oxen and dry cows.
" " " " " "	V	.22	.27	.23	.77	.03	.19	3.5	Mixed, mainly green..	5.1	Heifers, oxen and pregnant cows.
" " " " " "	VI	.14	.30	.14	.34	Trace	.08	2.2	Mixed, mainly brown..	5.0	Heifers, oxen and pregnant cows.
" " " " " "	VII	.14	.37	.18	.59	.03	.15	.33	Mixed, mainly brown..	5.0	Heifers, oxen and pregnant cows.
" " " " " "	VIII	.29	.32	.20	1.09	.02	.22	3.8	Mixed.....	4.5	Working oxen and pregnant cows.
" " " " " "	IX	.18	.33	.23	.66	.01	.18	3.7	Mixed.....	4.5	Working oxen and pregnant cows.

### MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE II (continued).

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub> .	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Crude Fibre.	Nature of Pasture.	Average Mgm. % P.	Animals bled.
Mimosa Farm, Umtata.....	IV	.31	.39	.12	.73	.13	.31	4.5	36.4	Mixed.....	4.6	Dry cows.
" " " " " "	V	.19	.29	.14	.85	.05	.21	4.3	37.6	Mixed.....	5.0	Dry cows.
" " " " " "	VI	.21	.32	.12	.67	.07	.30	4.8	—	Mixed, mainly brown.	4.0	Dry cows.
" " " " " "	VII	.26	.33	.16	1.07	.07	.30	6.3	37.2	Mixed, mainly brown.	3.0	Dry cows.
" " " " " "	VIII	.24	.25	.23	1.91	.04	.56	6.0	36.6	Mixed.....	4.1	Dry cows.
" " " " " "	IX	.23	.30	.14	1.18	.03	.38	5.4	36.8	Mixed.....	4.4	Dry cows.
Xura Tank Area, Lusitiski.....	IV	.16	.35	.27	.43	.09	.23	4.0	39.5	Mixed.....	5.0	Cows in calf, oxen and heifers.
" " " " " "	V	.25	.34	.26	.82	.16	.39	5.0	35.4	Mixed.....	4.8	Dry cows, heifers and oxen.
" " " " " "	VI	.24	.29	.21	.59	.18	.28	4.2	36.6	Mixed, mainly brown.	5.0	Dry cows, heifers and oxen.
" " " " " "	VII	.23	.31	.17	.81	.17	.33	4.7	38.8	Mixed.....	4.2	Dry cows, heifers and oxen.
" " " " " "	VIII	—	—	—	—	—	—	—	—	—	5.1	Dry cows, heifers and oxen.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Craddock Place, Port Elizabeth.....	IV	.26	.38	.35	1.86	—	—	10.3	33.6	Mixed, mainly green.	8.1	Oxen, dry cows and heifers.
" " " " " "	V	.26	.91	.34	1.51	1.18	1.51	10.8	33.8	Mixed, mainly green.	6.0	Oxen.
" " " " " "	VI	.39	.45	.20	1.73	.53	.71	10.1	33.0	Green.....	5.8	Oxen and dry cows.
" " " " " "	VII	.36	.31	.22	1.28	1.39	1.89	5.6	38.1	Mixed.....	—	—
" " " " " "	VIII	.32	.81	.26	.47	.47	.95	8.8	37.1	Mixed, mainly green....	—	—
" " " " " "	IX	—	—	—	1.90	—	—	—	—	—	—	—
Princeton, Bedford.....	IV	.36	.48	.18	1.60	.41	.68	6.8	36.4	Mixed, mainly green.	7.6	Heifers and oxen.
" " " " " "	V	.45	.82	.68	2.19	1.39	1.80	8.2	34.9	Mixed, and bushes....	6.0	Oxen.
" " " " " "	VI	.30	.62	.40	1.33	.78	.47	7.0	30.6	Mixed, mainly brown.	4.6	Oxen and dry cows.
" " " " " "	VII	.59	.81	.62	2.90	1.84	1.50	11.5	32.0	Mixed, and bushes....	5.4	Oxen and dry cows.
" " " " " "	VIII	.50	.60	.48	1.56	1.93	2.04	8.0	33.3	Mixed, mainly brown and bushes	4.7	Oxen.
" " " " " "	IX	—	—	—	—	—	—	—	—	—	—	—
Lombardspost, Bathurst.....	IV	.20	.31	.12	.69	.12	.28	5.3	29.2	Mixed, mainly green, short	6.3	Dry cows.
" " " " " "	V	.23	.36	.26	1.21	.48	.56	6.7	35.0	Mixed, mainly green.	3.8	Dry cows, and heifers.
" " " " " "	VI	.18	.36	.15	.55	.07	.16	5.0	19.9	Mixed, short.....	4.5	Dry cows, and heifers.
" " " " " "	VII	.47	.46	.17	1.72	.24	.42	11.9	27.7	Green, short.....	5.0	Dry cows, and heifers.
" " " " " "	VIII	.19	.33	.11	.82	.10	.31	4.1	31.6	Mixed, short.....	4.6	Dry cows.
" " " " " "	IX	.33	.77	.20	1.22	.28	.46	8.5	30.2	Mixed, mainly green..	—	—

TABLE II (continued).

Farm and District.	Survey.	P <sub>2</sub> O <sub>5</sub> .	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.	Cl.	Crude Protein.	Crude Fibre.	Nature of Pasture.	Average Mean % P.	Animals bled.
Kingston, Albany.....	IV	.37	1.45	.26	1.70	.26	.47	11.5	32.3	Bushes.....	7.8	Heifers.
Woodvale, Albany.....	V	.31	2.33	.94	1.51	.60	.55	12.0	23.6	Bushes.....	5.8	Dry cows.
" ".....	VI	.36	2.82	1.22	2.25	1.25	1.11	9.9	42.6	Mainly bushes and mixed, short	3.9	Dry cows.
" ".....	VII	.58	1.50	.86	2.90	1.06	.59	14.8	24.8	Mainly bushes and mixed, short	3.7	Dry cows.
" ".....	VIII	.31	.35	.20	1.30	.18	.72	9.0	27.4	Green, short	5.3	Dry cows.
" ".....	IX	.36	1.67	1.07	2.42	.90	1.00	10.1	24.6	Mixed, mainly green and bushes.	6.2	Dry cows.
Allandale, Middelburg, C.P.....	IV	.46	.67	.23	1.66	.10	.28	6.5	29.7	Green, short and bushes	5.6	Lactating cows.
" ".....	V	.39	.62	.25	1.66	.04	.23	8.7	36.1	Mixed, mainly green and bushes	5.1	Dry cows.
" ".....	VI	.25	.49	.18	1.17	Trace	.08	5.3	41.0	Brown, and bushes...	5.2	Dry cows and heifers.
" ".....	VII	.25	.49	.21	1.29	.04	.18	7.4	40.3	Mixed, and bushes...	3.5	Dry cows and heifers.
Leeuwfontein, Middelburg.....	VIII	.25	.89	.28	1.30	.03	.20	3.0	33.6	Green, and bushes...	5.3	Dry cows and heifers.
" ".....	IX	.31	1.08	.33	1.70	.02	.34	9.7	32.7	Green, and bushes...	5.4	Dry cows and heifers.
<i>Orange Free State.</i>												
Biabops Glen, Bloemfontein.....	IV	.38	.63	.25	.98	.02	.14	4.6	32.5	Mixed, and bushes.	5.3	Dry cows.
" ".....	V	.26	.41	.31	1.13	.03	.20	6.0	35.2	Mixed, mainly green...	4.6	Dry cows.
" ".....	VI	.14	.26	.22	.54	Trace	.05	2.8	38.8	Brown.....	4.9	Dry cows.
Biabops Glen, Bloemfontein.....	VII	—	—	—	—	—	—	—	—	—	—	—
" ".....	VIII	.34	.93	.33	1.86	.05	.43	7.3	39.2	Green, and bushes...	—	Dry cows.
" ".....	IX	—	—	—	—	—	—	—	—	—	—	—
Naseby Thoms, Kroonstad.....	IV	.26	.53	.24	1.35	.02	.34	5.2	32.6	Mixed.....	6.1	Dry cows.
" ".....	V	.29	.36	.32	1.57	.02	.42	6.6	34.4	Mixed, mainly green...	5.7	Dry cows.
" ".....	VI	.22	.41	.29	1.09	Trace	.15	4.3	36.0	Brown, long.....	4.9	Dry cows.
" ".....	VII	.19	.34	.19	.36	.02	.07	3.5	36.5	Brown, long.....	5.0	Dry cows.
" ".....	VIII	—	—	—	—	—	—	—	—	—	—	—
Naseby Thoms, Kroonstad.....	IX	.20	.46	.24	.90	.01	.28	6.6	32.8	Mixed, mainly brown...	5.0	Dry cows and oxen.
The Outlook, Bethlehem.....	IV	.36	.36	.21	2.22	.03	.39	9.2	31.5	Green, short.....	5.5	Lactating cows.
" ".....	V	.25	.34	.20	1.11	.04	.22	3.3	34.4	Mixed, mainly brown...	3.8	Dry and lactating cows.
Sebastopol, Bethlehem.....	VI	.09	.27	.13	.71	Trace	.11	3.0	37.4	Brown.....	—	—
" ".....	VII	.11	.26	.11	.13	.02	.05	3.3	36.9	Mixed.....	4.5	Pregnant cows.
The Outlook, Bethlehem.....	VIII	.25	.21	.16	1.75	.07	.37	6.8	33.9	Mixed, mainly green...	2.4	Lactating cows.
" ".....	IX	—	—	—	—	—	—	—	—	—	—	—

The description of the pasture samples is superficial and based on the following:—

“Mixed” means that the sample can be divided into approximately equal quantities of green and brown herbage respectively. A sample labeled “mixed, mainly green” contains 60-90 per cent. green herbage, while “mixed, mainly brown” indicates the presence of 60-90 per cent. brown herbage. In a “brown” sample the presence of not more than 10 per cent. of green material is allowed, while in a “green” sample the amount of brown material should not exceed 10 per cent. These terms refer to grasses only, and if bushes or shrubs are present mention of these is made accordingly. The blanks in the table indicate that for the period in question no collections were made. Outbreaks of infectious diseases occurred on several occasions, during which periods primary claim was made upon the services of the Government veterinary officers for assistance so that they could not always carry out the collections of samples regularly.

The analyses of the samples obtained from Kuruman and Mafeking given in Table II are grouped with those from centres in the Province of the Transvaal on account of the proximity of these districts to the latter province. Strictly speaking these areas lie in the Cape Province.

Generally speaking, values for blood phosphorus above about 4.5 mgm. per 100 c.c. blood are not in any way indicative of phosphorus deficiency but as many classes of stock including dry and lactating cows, oxen, heifers and sheep were bled for analysis this figure is by no means exact but could serve to show up deficiencies and sufficiencies of phosphorus at the time of bleeding. For more details in regard to the relation between the phosphate content of the pasture and that of the blood the reader is referred to the first report on this investigation (du Toit *et al.*, 1933). Another factor which is brought out by the values for blood phosphorus in the table above is the apparent lack of agreement between the phosphorus content of the pasture and that of the blood in quite a number of cases. Although earlier work has undoubtedly established a direct relation between phosphorus intake and blood phosphorus it does not follow that any one determination of the percentage phosphorus in pasture should be reflected directly in the inorganic phosphorus content of the blood and that for two reasons mainly. Firstly, the pasture collected may not represent a true sample of that eaten by stock on account of the method of collection employed for the above surveys, and secondly the percentage value of phosphorus in pasture gives no indication of the amount of pasture available of that composition, i.e. of the total intake of the animals on such pasture. Obviously, not much available pasture containing high phosphorus, as for instance at the beginning of a new season's growth will not show as high a figure for inorganic phosphorus in blood as abundant pasture of average composition. Hence information of a more exact nature in regard to the relation between blood and pasture phosphorus and also in regard to phosphorus deficiency or otherwise can be obtained if both values are studied for periods as can be done in the above table, rather than for corresponding months only. If this is done it will be noticed that the higher values for pasture

phosphorus tend to be reflected as higher values for blood phosphorus, e.g. Barberton, Zoutpansberg, Nongoma, Pietermaritzburg, Port Elizabeth, etc. However, the agreement is by no means of the nature of that obtained in controlled experiments and is being further investigated in a few areas only where true samples of the pasture eaten are collected by following animals and bleeding them afterwards for blood samples for analysis. Furthermore, the analytical values of pasture collected on the basis reported in this article are being compared with those obtained from true samples of pasture eaten gathered by following animals as already stated.

One point is nevertheless evident from a study of the values for blood phosphorus when compared with the phosphorus content of the pasture, viz., that practically no values are indicative of a phosphorus sufficiency in South African pastures throughout the year, although quite a fair number of both pasture and blood analyses suggest that during seasons of active growth and highest rainfall (mainly in summer) the pasture probably contains enough phosphorus for the requirements of non-lactating stock.

The phosphorus contents of the pasture samples corroborate the statements made in the first report of this series (1932) and may be summarized as follows: (a) The percentage phosphorus in pasture decreases as the plants mature and extremely low values are often obtained during winter in cases where only fully grown out grasses are available. These values tend to be even lower after adverse conditions of temperature, such as frost. (b) Of the climatic conditions during the growing seasons rainfall apparently determines the stage of growth of pasture and hence its phosphorus content. (c) Very abundant rains do not appear to favour a high phosphorus content of pasture, while the question of a possible leaching effect of the rains may be a possible explanation but is still a matter of conjecture, e.g. Derby 56, Piet Retief; Stainton, Ixopo; Mimosa Park, Potchefstroom, etc. (d) Pasture samples containing shrubs invariably show higher values for phosphorus than those consisting of grasses only. (e) Deplorably small amounts of phosphorus are present even during the best seasons of the year in samples from certain areas, e.g. Melbourne, Port Shepstone, Marico, Mafeking, Kuruman, etc., and in some other areas during the winter months. It is inconceivable that the phosphorus requirements of animals could be met under such adverse conditions. (f) The supplementation of pasture used for grazing by phosphates for the greater part of the year is essential if maximum production is aimed at.

The rest of the constituents given in Table II do not present such a gloomy picture as the phosphate values do. Crude protein is certainly very low at times and as stated in the first article can hardly be believed to satisfy the protein requirements of the animals at all seasons of the year. Appreciable increases in the protein content of the pasture is associated with the presence of new growth and naturally an increase in the phosphorus content. Low phosphorus and low protein, usually indicative of an advanced stage of growth of the pasture, are on the whole associated with high values for crude fibre. Lime values for some pasture samples are distinctly on the low side but the values that need be viewed with concern are those for sodium. Generally it appears that the pastures

are extraordinarily low in sodium and even with moderate milk production it is hardly conceivable that the daily pasture ration satisfies the sodium requirements of a lactating cow. As a matter of fact in a number of cases the sodium content of 25 lb. of dry pasture, of the type represented by the samples—the approximate daily intake of a 1,000 lb. cow—is less than the sodium actually secreted in two gallons of milk. The question of a possible sodium deficiency in South African pastures and the effect on animals is being investigated at the present time and will be reported on in due course.

If the samples analysed are at all representative of South African pastures the latter must be looked upon as distinctly poor, for the greater part of the year, for purposes of production in stock farming. Supplementary feeding, or, alternatively, the necessity for improvement of the pastures, be it by fertilizing or better management to increase their feeding value, is strongly indicated.

### SUMMARY.

1. The third report of the series on the mineral content and feeding value of South African natural pastures is presented.

2. Both pasture and blood analysis confirm the earlier statement that practically all South African pastures are low in phosphorus for the greater part of the year.

3. Crude protein varies more or less directly as the phosphorus content of the pasture and is extraordinarily low during certain seasons of the year.

4. Generally, the sodium content of the samples is so low that it suggests an insufficiency of sodium for producing animals on pasturage.

5. The samples analysed were representative of the herbage in the areas of collection and not necessarily of the pasture actually eaten by stock as will be the case with all samples collected subsequently and to be reported on in due course.

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## **A Study of the Mineral Content and Feeding Value of Natural Pastures in the Union of South Africa.**

### **IV. The Influence of Season and Frequency of Cutting on the Yield, Persistency, and Chemical Composition of Grass Species.**

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##### **II. Successive monthly cuts.**

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## INTRODUCTION.

THE mineral surveys of natural pastures in the Union of South Africa inaugurated in May, 1930, and on which a first report was published by du Toit and associates (1932), have been greatly extended during the last two years. At the present time more than 200 samples of pasturage as grazed by the animals are being collected every month from chosen farms distributed all over the country. In order to interpret correctly data so obtained it is necessary to study the seasonal variations in chemical composition of individual species of indigenous grasses under controlled or known conditions at fixed stages in their vegetative growth because of the known variability in mineral and protein content as growth advances towards maturity.

Furthermore such a study provides data on the feeding value of individual species of grasses which compose pastures and therefore on the economic utilization of such pastures which is often attainable in practice by bearing in mind the relation between feeding value and stage of growth. This relation is of course of paramount importance when the problem of pasture research is considered from the practical aspect of pasture improvement. Stage of growth determines palatability perhaps to a greater extent than any other single factor and likewise also feeding value—both factors which cannot be lost sight of when the object of pasture improvement, viz., to provide more and better grazing for the animal is considered. The investigation of the chemical composition of individual species of grasses at various stages of growth as reported in this article was therefore undertaken as a supplementary study to the determination of the feeding value of South African pastures (du Toit *et al.* 1932; 1935). This study will effect the accumulation of data on fundamental aspects of pasture improvement such as feeding value in relation to growth, digestibility as affected by the maturity of the pasture, light which chemical analysis may throw on animal production on pasture, etc. In short, the plot work was undertaken because it creates an opportunity of introducing the animal factor into pasture studies. Hence factors which affect the feeding value of pastures for the animal, as for instance stage of growth, productiveness of the pasture, palatability, digestibility, etc., are being stressed in this investigation of which the present article may be considered as something of the nature of a progress report.

The plots under consideration in this article were too small to study yield conclusively and larger plots have subsequently been established, but it was considered well worth the extra work to weigh all the samples obtained from the different frequencies of cutting and to study these weights in conjunction with the chemical data with the object of arriving at tentative conclusions in respect of species of grasses which could with advantage be studied on larger plots at a later date.

The investigation on the species established in plots was initiated in February, 1932, and a preliminary report was published by du Toit *et al.* (1934). In the present paper it is proposed to deal with the results obtained from a further 12 months' work on the same grass species in conjunction with the data from the previous year.

TABLE I.

Date.	Rainfall in inches.	Showers of note with dates in brackets.	Average hours sunshine.	Average maxi- mum tempera- ture in °F.	Average mini- mum tempera- ture in °F.
Feb., 1932	4.85	0.66 (2), 1.05 (4), .30 (5), 1.40 (17), 0.43 (19), 0.20 (25), 0.48 (28)	7.7	86.0	61.0
March, 1932	2.08	1.58 (9), 0.29 (20).....	6.9	84.0	58.0
April, 1932	0.77	0.17 (23), 0.23 (24), 0.31 (26)....	8.1	82.0	49.0
May, 1932	0.15	—	8.6	75.0	40.0
June 1932	Nil	—	8.4	73.0	34.0
July, 1932	Nil	—	9.0	71.0	30.0
Aug., 1932	Nil	—	9.6	77.0	36.0
Sept., 1932	0.88	0.87 (20).....	8.8	82.0	49.0
Oct., 1932	1.76	0.88 (3), .58 (4), 18 (28).....	8.1	84.0	56.0
Nov., 1932	2.54	0.72 (7), 0.22 (10), 0.28 (19), 0.40 (22), 0.24 (25), 0.37 (30)	8.5	81.0	59.0
Dec., 1932	3.54	0.50 (4), 0.56 (14), 0.35 (16), 0.54 (24), 1.30 (26)	7.4	87.0	61.0
Jan., 1933	1.54	1.06 (15).....	8.2	89.0	61.0
Feb., 1933	0.70	0.29 (19), 0.36 (28).....	8.5	91.0	60.0
March, 1933	2.06	0.32 (1), 0.90 (3), 0.42 (8), 0.34 (15)	8.6	88.0	55.0
April, 1933	1.05	0.85 (2), 0.16 (18).....	8.8	78.0	50.0
May, 1933	0.07	—	8.8	82.0	41.0
June, 1933	0.35	0.35 (22).....	8.0	67.0	32.0
July, 1933	0.01	—	8.0	70.0	36.0
Aug., 1933	Nil	—	9.0	70.0	40.0
Sept., 1933	0.42	0.42 (1).....	9.0	75.0	46.0
Oct., 1933	0.46	0.11 (26), 0.25 (27), 0.10 (28)...	9.0	90.0	54.0
Nov., 1933	14.55	Evenly distributed over month, 10 days on which no rain fell	6.7	81.0	59.0
Dec., 1933	6.20	2.36 (13), 0.24 (14), 1.00 (15), 1.40 (26), 1.08 (31)	7.5	83.0	60.0
Jan., 1934	7.54	2.02 (2), 1.15 (13), 0.45 (16), 2.00 (29), 0.68 (30)	7.0	85.0	62.0
Feb., 1934	2.75	0.23 (3), 0.21 (8), 0.69 (9), 0.68 (13), 0.28 (19), 0.58 (23)	7.4	84.0	63.0
March, 1934	1.43	0.15 (11), 0.31 (25), 0.97 (26)....	7.5	86.0	58.0

In March, 1933, the work was extended to include eleven additional species, newly established, and the results of their analyses for the period March, 1933, to February, 1934, will be discussed in a separate section of this report. The method of establishing the grasses and of taking monthly, two-monthly, etc., samples was the same as that described in the first report on the plot experiments, and will not be dealt with here. The first samples were taken on the 28th March, 1933, and then on the same date of each succeeding month until February, 1934.

All the new grasses were well established towards the end of 1932 or early in January, 1933. As stated in the previous paper the grasses received no further attention except for occasional weeding. The work was, therefore, done throughout on grasses subjected to the prevailing climatic conditions.

## METEOROLOGICAL DATA.

The outstanding features in the climatic conditions for the period February, 1932, to January, 1933, have already been indicated (du Toit *et al.*, 1934). Mention need only be made of the fact that it was a period of exceptionally low rainfall, a total of 17.43 inches, which is about 12 inches below normal, being recorded. Table I gives a summary of the general meteorological conditions for the full period under discussion. It was thought advisable to give not only the total but also the approximate distribution of the rainfall for every month.

The readings were taken at the Onderstepoort Veterinary Institute at a distance of about half a mile from the experimental plots and may be considered therefore to represent the rainfall at the actual site of the experiment.

The rainfall for the latter half of the growing season 1932-33 remained low, two light showers towards the end of February, fairly well distributed precipitations for the first half of March and a good shower on the 2nd April being recorded. The usual dry period followed except for an isolated shower in mid-winter when rain has generally very little effect on growth, on account of the low day and night temperatures.

No falls of note were registered until the beginning of November (1933) which was a month of exceptionally high rainfall. There were only 10 days on which no rain fell and over 14 inches were recorded for the month. The average daily hours of sunshine and maximum temperatures dropped from 9.0 and 90.0 in October to 6.7 and 81.0 respectively in November, while an increase for both these growth factors were noted for December. There were no significant changes in the average daily hours of sunshine and maximum temperatures during January and February, 1934. The average minimum temperatures increased steadily from 54.0° F. in October to 63.0° F. in February.

During December and January good average rainfalls were registered while the figure for February dropped to only 2.75 inches. March was comparatively dry. Precipitations of 0.31 and 0.97 inches were recorded for the 25th and 26th respectively, but these showers had very little effect on the herbage cut on the 28th of March. The average maximum temperature was slightly higher than during the preceeding months.

For the period November, 1932, to February, 1933, the rainfall was in marked contrast to that recorded for the period November, 1933, to February, 1934, only 8.3 inches being registered for the former period while during the latter period heavy downpours totalling 31.0 inches occurred.

## SECTION 1.—OLD ESTABLISHED SPECIES.

### I. MONTHLY CUTS.

The number of monthly cuts obtainable from plants growing under natural conditions is necessarily largely dependant upon the prevailing climatic conditions. The growing season in this part of

the country commences with the first rains, usually in September, and terminates with the cessation of summer rains in about April. The discussion of the chemical composition and yield of herbage cut at monthly intervals is therefore limited to the available data for the growing seasons falling within the experimental period.

Of the eleven species on which work was started in February, 1932, one, *Rhynchelythrum roseum*, died completely during the winter of 1933.

Table A in the appendix gives the dates of cutting, the yield of dry matter,\* and the percentage composition of the dry matter of each cut, together with a short description with regard to the stage of growth, of the monthly cuts for the period February, 1933, to March, 1934, for the ten remaining species to be dealt with in this section of the paper.

The composition of the dry matter of the monthly cuts of one and the same species does not vary markedly, and if a general survey of these figures for all the grasses is made it will be noted that the percentages of individual constituents from month to month tend to fluctuate in a manner similar for all grasses. Consequently, month to month averages for all the species combined was decided upon as a basis for discussion rather than a consideration of the fluctuations of each grass separately. A study of Table A in the appendix reveals the fact that the deviations from such a generalisation, due to differences in response to climatic conditions by individual species are not numerous, although not entirely absent.

The average yield and composition of the dry matter of the ten species from month to month for the whole experimental period is given in Table II below. For the purpose of bringing out the seasonal variations more clearly the data are put into graphical form in Fig. I.

#### *Discussion of results of monthly cuts.*

##### *(a) Season 1931-32.*

Due to the fact that the investigation was begun in February, 1932, data for only three monthly cuts are available for this growing season. A feature in the composition of the dry matter for the three months, February, March, and April, is the inverse relationship between crude protein content and crude fibre content, the latter constituent shows a downward trend from February to April, whereas crude protein rises from February to March with a further slight increase in April. These results are in harmony with work carried out by Archibald and associates (1932) at the Massachusetts Agricultural Experiment Station, U.S.A. The average daily maximum temperatures decreased from February to April while the average daily amount of sunshine was actually higher in April than in either February or March. It cannot, therefore, be said that the lower figure for crude fibre in April, as opposed to the values for February

\* All analyses in this paper are given as percentages on absolutely dry basis.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

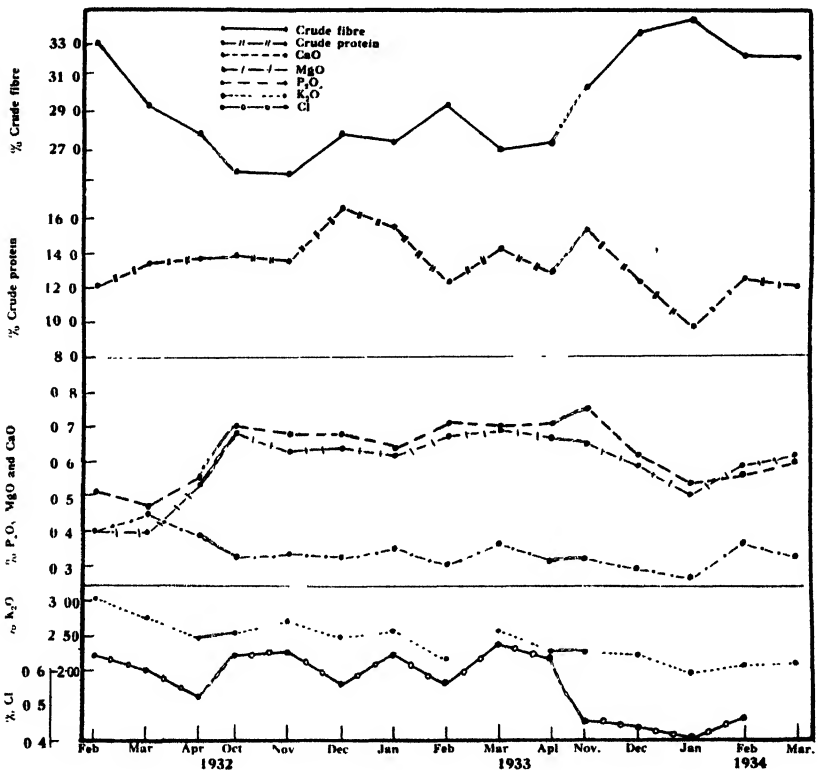
TABLE II.

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble Ash.	Crude Fibre.	Total yield in gms.
February, 1932.....	.41	11.9	.51	3.01	.40	.18	.64	—	33.0	300.0
March, 1932.....	.46	13.4	.47	2.76	.40	.14	.60	—	29.5	200.0
April, 1932.....	.39	13.7	.59	2.41	.54	.15	.52	—	28.1	90.0
October, 1932.....	.33	13.8	.70	2.50	.68	.24	.64	—	25.9	65.0
November, 1932.....	.34	13.7	.68	2.72	.63	.16	.65	—	25.6	63.0
December, 1932.....	.33	16.6	.68	2.45	.64	.15	.56	—	28.1	82.0
January, 1933.....	.35	15.3	.64	2.57	.63	.19	.64	—	27.5	75.0
February, 1933.....	.30	12.3	.70	2.17	.67	—	.56	—	29.5	40.0
March, 1933.....	.36	14.1	.69	2.55	.69	.20	.67	—	27.1	60.0
April, 1933.....	.31	12.8	.71	2.25	.67	.19	.63	—	27.5	46.0
November, 1933.....	.32	15.4	.75	2.26	.65	.37	.45	4.93	30.6	136.0
December, 1933.....	.29	12.4	.62	2.21	.59	.31	.43	4.31	33.7	140.0
January, 1934.....	.27	9.8	.54	1.96	.50	.16	.40	3.85	34.4	160.0
February, 1934.....	.37	12.5	.57	2.06	.59	.15	.46	4.45	32.3	80.0
March, 1934.....	.33	12.0	.60	2.12	.62	—	—	4.41	32.2	50.0

and March is attributable to a diminution in amount of sunshine (Archibald *et al.*, 1932), rather may it be interpreted that less lignification had taken place in the April sample than in the February and March samples, or, in other words that the grass was at an earlier stage of growth in April than in February or March (c.f. Table B in appendix of previous paper).

Phosphate and lime show opposite tendencies in that the peak value for the former constituent in March corresponds with the lowest figure for lime at the same date, a finding in agreement with

Fig. 1.



Woodman's (1926) conclusions in this respect. Reference to Table I giving the general meteorological conditions shows that the rainfall decreased from February to April. The amount of growth decreased similarly over this period (c.f. Table 2) while, as stated above, the April samples were at an earlier stage of growth than the February or March samples. In view of the findings of Richardson and associates (1931), viz. that nitrogen assimilation and the absorption of essential nutrients are most active at the tilting stage and that photosynthesis rises to a maximum shortly after the flowering stage the lower value for phosphate in the April sample as opposed to those for February and March is rather surprising. It is difficult at this stage to discuss the percentage composition of the samples in relation to soil moisture as is done by Richardson and associates (*loc. cit.*).

Such considerations do not fall within the scope of this article but it may be pointed out that the low level of soil saturation which no doubt existed during April may be responsible for a relatively low figure for phosphate corresponding with high values for lime and crude protein in the April herbage. (Richardson, *loc. cit.*) Mention should also be made of the low values obtained for  $P_2O_5$  in the green leaves of Bechuanaland grasses by Henrici (1928) during periods of summer drought and the finding of this author that "there is a decided tendency to lower values towards autumn".

The potash and chlorine series of values in Table II both show downward tendencies from February to April while magnesia varies in a manner similar to lime.

(b) *Season 1932-33.*

The exceptionally dry weather conditions which characterised this growing season have already been referred to, the best rainfalls having been registered during December.

The inverse relationship between crude fibre and crude protein contents indicated for the period February to April, 1932, is again apparent for the latter half of the present growing season. The crude fibre tends to increase from October to February reaching its highest level for the whole period in the dry matter of the herbage cut at the end of the latter month. The average daily maximum temperatures vary similarly, the highest figure being recorded for February while at the same time this month was the driest for the season with a total rainfall of only 0.70 inches. Crude protein rises to a maximum for the season in December and then falls to a minimum in February. Phosphate shows little variations from month to month and tends towards a minimum in February and a maximum for the season in March.

Phosphate and lime again fluctuate in opposite directions throughout the whole season, the lowest and highest figures for the latter constituent occurring in January and April respectively.

A remarkable feature in the composition of the dry matter for this season is the consistently high values for lime in comparison with figures for phosphate, that must be looked upon as distinctly low. At no time does the phosphate level rise above the lowest figure for the previous season while the values for lime never fall below the highest figure obtained in April, 1932.

Magnesium like lime remains fairly constant with a minimum in January and then rising steadily to a maximum in March. The figures for this constituent remain, as in the case of lime, higher than the maximum for the previous season.

The graphs for the potash and chlorine values reveal similar changes for the period under discussion, slightly lower values for both constituents being obtained in February.

In view of the fact that the grasses comprising the crop for February were largely at an immature stage of growth (c.f. Table A in the appendix) it is interesting to note that the maximum for crude

fibre and minima for phosphate, crude protein and potash for the entire growing season occur in the herbage for this month. It was undoubtedly the driest month of the season, the shower of 0.29 inch registered on the 19th being the only rainfall for the month preceding the February cut. Such dry conditions would no doubt cause wilting of the young immature herbage and it is possible that the aerial parts lose water not only by excessive transpiration but by the roots as well. Water thus lost by the latter channel may carry certain salts which have not been organically bound, in solution, thus causing a migration of nutrients from the aerial parts to the soil (see Henrici 1928).

(c) *Season 1933-34.*

Practically no growth took place after the light showers of September and October with the result that the first monthly cuts for this season were taken at the end of November.

The herbage for November was mainly in an immature stage of growth; in the case of a few species only did flower-heads begin to appear. Exceptionally high values were obtained for the crude protein content of most species, especially *Urochloa pullulans* and *Panicum maximum*.

As stated previously copious rains fell during November but the amount and intensity of sunshine were less than during the previous and succeeding months. Climatic conditions appear to have been more favourable for rapid growth during December and January when the combined rainfall for the two months was slightly less than the amount registered for November alone, but, nevertheless apparently sufficient to maintain a high level of soil moisture saturation. The result was that the composite herbage crops for December and January attained a higher degree of maturity than that for November.

Following upon a rapid decline in the rainfall from January to March the amount of growth decreased similarly. As a matter of fact growth practically ceased after the March samples were taken, only three species showing sufficient growth at the end of April for the usual monthly cuts.

The average composition of the dry matter of the composite monthly cuts from November to March is in close agreement with the stage of growth attained by the grasses. Generally speaking, all the inorganic constituents and crude protein fall from maxima in November to minima in January followed by secondary peak values in February when climatic conditions favoured slower growth than in January (see Table A).

Crude fibre, on the other hand, shows an opposite tendency, the lowest figure was obtained in November after which the value for this constituent steadily rose to a maximum in January. The inverse relationship between crude fibre and crude protein content, which was shown to exist during the two previous growing seasons, is again in evidence, while the variations in the average values for the latter



constituent and phosphate display a close parallelism throughout the present growing season. The comparatively low values for these two constituents in the monthly cuts for January are worthy of note.

If a general survey of the data for the whole experimental period is made one is forced to the conclusion that in the absence of soil and climatic deficiencies stage of growth is the main factor influencing the mineral and protein content of pasture species. The effect of soil composition on the composition of herbage plants does not fall within the scope of this investigation while the data do not allow of definite conclusions in respect of the influence of climatic factors except in so far as they effect stage of growth.

Mention may however be made of the fact that phosphate values are low during periods of dry weather. This observation, if confirmed, may perhaps be explained on the basis of Richardson's findings that low soil moisture tends to produce herbage of a low phosphate content.

In any case, the composition of monthly cuts of herbage cannot be looked upon as markedly different from one another. During some months a slightly more or less advanced stage of growth is attained by a species when compared with its growth of the previous month and resulting in a slight decrease or increase in the percentage content of the constituents, but, on the whole, the monthly growths of the grasses studied must be looked upon as herbage of a comparatively high composition at a comparatively early stage of growth.

#### *(d) Yield and Persistency of the Individual species.*

It has been pointed out previously that the area cut was too small to study yield conclusively. If it is, however, remembered that the grasses were planted uniformly in rows containing about twenty plants each and that always the same row was cut in order to obtain monthly or two-monthly samples, as the case may be, it will be admitted that the production of dry matter by the twenty plants in the first row of one species during any one month is comparable with the production of dry matter by the same twenty plants during any other month of the year on condition that none of their vital functions have been adversely affected by the severe treatment.

In view of the fact that the area cut was small, as already stated, and that the weights obtained were not based on duplicate plots too much importance cannot be attached to the yield figures, especially when the yield from the first row of one species is compared with that from the first row of another species, but one is justified in drawing certain broad inferences for further investigation in respect of the relative yielding capacities of individual species subjected to a system of cutting at monthly intervals *under the climatic conditions obtaining during the experimental period.*

Table III below gives the total yield in grams from month to month as well as the gross yield for the whole of the experimental period February, 1932, to March, 1934, for each of the ten species studied.

TABLE III.  
(Yields in grams.)

	<i>Pennisetum ciliare.</i>	<i>Eragrostis superba.</i>	<i>Amphilo- phis insculpta.</i>	<i>Cynodon dactylon.</i>	<i>Setaria gerrardii.</i>	<i>Urochloa pullulans.</i>	<i>Hypar- rhena hirta.</i>	<i>Panicum maximum.</i>	<i>Cymbopogon plurinoides.</i>	<i>Themeda triandra.</i>
February, 1932.....	390	125	497	330	355	353	307	285	215	185
March, 1932.....	230	200	260	170	210	320	250	170	110	70
April, 1932.....	120	100	130	95	100	100	120	70	60	—
October, 1932.....	80	150	—	50	70	55	45	15	75	115
November, 1932.....	100	130	65	30	75	50	35	20	60	50
December, 1932.....	160	135	75	45	115	75	65	30	65	55
January, 1933.....	205	105	85	40	95	40	55	35	45	45
February, 1933.....	100	90	85	35	25	—	25	—	17	20
March, 1933.....	135	125	75	35	45	45	50	35	20	25
April, 1933.....	85	85	55	60	45	20	45	20	15	25
November, 1933.....	247	120	60	146	39	50	44	50	330	280
December, 1933.....	216	178	200	42	58	83	100	326	80	120
January, 1934.....	350	190	104	352	55	100	132	160	52	96
February, 1934.....	135	140	50	188	56	77	60	46	52	20
March, 1934.....	220	104	10	—	76	26	28	8	9	—
TOTALS.....	2,773	1,977	1,751	1,618	1,419	1,394	1,381	1,270	1,205	1,106

In considering the relative yields of these grasses it should be borne in mind that they received no further attention after being well established towards the end of 1931. The grasses were selected from locally growing species and the soil on which they were planted was of a type that is common in this area, viz., a sandy loam of fine texture. Any changes in the general condition of the plants during the experimental period may therefore be ascribed to the effect of the treatment, i.e. cutting at regular intervals, under the prevailing climatic conditions.

It will be both necessary and interesting to give at this stage short descriptions of the plots of individual species as to their state in January, 1934, a few months before the conclusion of the experimental period under consideration:—

*Hyparrhenia hirta*.—About 50 per cent. of the plants in the first row (monthly cut) are dead.

*Themeda triandra*.—Approximately 50 per cent. of the plants in the first row, 25 per cent. in the second and a few plants on the remainder of the plot are dead.

*Cymbopogon plurinoides*.—In each of the first and second rows about 25 per cent. of the plants perished.

*Cynodon dactylon*.—The individual plants have developed into a uniform sward covering the whole plot.

*Amphilophis insculpta*.—About 50 per cent. in the first row and roughly 25 per cent. of the plants in the second row are dead.

*Eragrostis superba*.—Only about 10 per cent. of the plants in the first row are dead.

*Setaria Gerrardii*.—Generally speaking, 25 per cent. of the plants on the whole plot are dead.

*Panicum maximum*.—Of the plants in the first row about 50 per cent. and approximately 10 per cent. on the remainder of the plot are dead.

*Crochloa pullulans*.—Of the first row only about 25 per cent. of the plants are dead.

*Pennisetum ciliare*.—With the exception of *Cynodon dactylon* this is the only plot where none of the plants established towards the end of 1931 died.

To summarise briefly, the plants in the first rows of the majority of the species suffered severely as a result of the treatment, in a few cases the second rows (two-monthly cuts) were badly affected while the number of plants which died in the third to the twelfth rows was negligible for the majority of the grasses. The plants subjected to lenient systems of cutting developed generally to fair-sized tufts, but the rows remained, with the exception of *Cynodon dactylon*, easily distinguishable from one another.

That plants in the first rows were dying was observed for the first time after the winter of 1932. It is obvious therefore that the results given in Table III are comparable only under the specific

conditions of this experiment and the weights thus indicate the monthly and total yields obtainable from each species when subjected to a system of cutting at monthly intervals under the prevailing climatic conditions. In the absence of climatic deficiencies or under less severe treatments the behaviour of individual species would probably have been different from what the results tend to suggest. But, at the same time, it is just such unfavourable climatic conditions that the natural pasture is subjected to under conditions of practical farming in this country.

The data in Table III undoubtedly suggest that of the ten species studied *Pennisetum ciliare* stands above the others in that it produced the greatest gross weight of dry matter and definitely proved itself to be one of the most drought resistant grasses, *Cynodon dactylon* being the only other grass which was not visibly affected by either drought or the treatment. *Eragrostis superba* takes second place as a producer of dry matter and was observed to be highly resistant to drought and artificial close-grazing. *Amphilophis insculpta* is third followed by *Cynodon dactylon* and *Setaria Gerrardii*. The latter grass lacks the persistency of the other species in that not only the first row, which was subjected to the system of cutting at monthly intervals, but the whole plot was affected by the treatment. *Urochloa pullulans* and *Panicum maximum*, while proving to be excellent grasses in respect of chemical composition, take only sixth and eighth places, respectively, as producers of dry matter. These two grasses, especially the *Panicum* species, do not stand up so well against drought and close-grazing as is the case with the *Pennisetum* and *Cynodon* species, but may be expected to compare more favourably with *Pennisetum ciliare* in respect of productivity if climatic conditions are more favourable. *Hyparrhenia hirta* produced approximately the same gross weight of dry matter as *Urochloa pullulans* but proved to be less resistant to the treatment and is of inferior quality as a stock feed. *Cymbopogon plurinoides* and *Themeda triandra* take last places in this method of deciding the yield of pasture species, both grasses appear to thrive best during the first half of the growing season, namely in October or November.

(c) *The effect of climatic conditions on yield.*

Owing to the size of the area cut and the harmful effects of the treatment under the adverse climatic conditions on the stand of grass in the first rows, it is not possible to make strict comparisons between the yields obtained for the various growing seasons falling within the experimental period. However, the influence of climate on the productivity of grasses during any one season may be indicated.

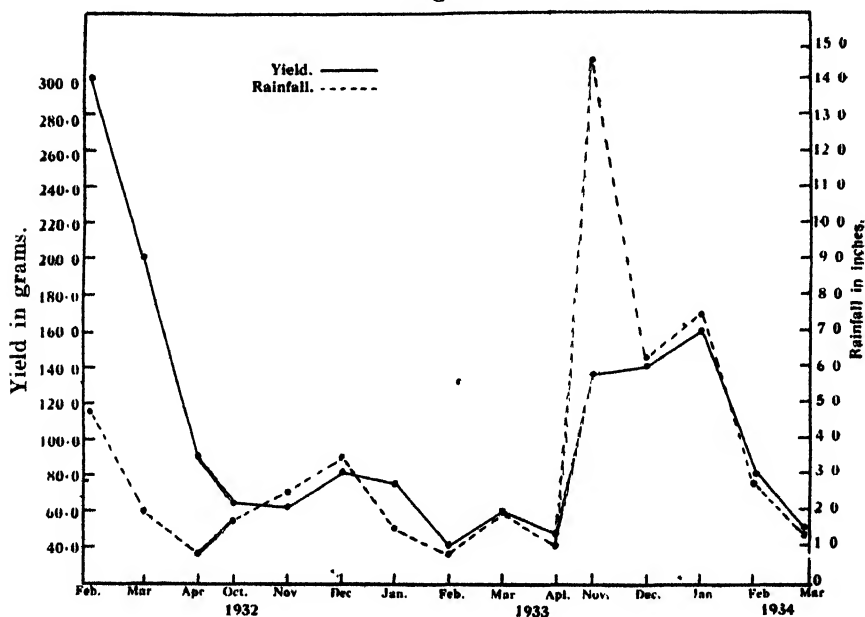
For the purpose of estimating the effect of rainfall on the yield of dry matter the average weights in Table II will be considered in relation to the rainfall data given in Table I. In order to bring out the influence of rainfall more clearly the relevant data are put into graphical form in Fig. 2.

With few exceptions the yield and rainfall curves fluctuate in a similar manner for the three seasons concerned. The average production of dry matter dropped from 300 grams in February, 1932, to

only 90 grams in April of that year, while the monthly rainfall decreased from 4.85 to 0.77 inches during the same period. The highest yield for the whole experimental period was obtained at the end of the first month of the investigation. This may largely be ascribed to the fact that none of the plants were as yet affected by drought or treatment.

The yields for the 1932-33 season were extremely low throughout, the best crop (82 grams) being obtained in December coinciding with the highest precipitation for the period. The low production figures were no doubt due in part to the fact that several plants were already dead at this time, but may mainly be attributable to the unfavourable climatic conditions, namely inadequate rainfall consisting mainly of light showers at infrequent intervals coupled with high temperatures.

Fig. 2.



There is an apparent lag in the effect of the rainfall on the yield for January. Reference to Table I reveals the fact that a fall of 1.30 inches was registered on the 26th of the previous month, two days before the herbage for that month was cut. That the yield curve does not drop in sympathy with the rainfall curve from December to January is therefore attributed to the fact that the precipitation referred to above could only have exerted its influence on growth during the early part of January.

The average productivity of the grasses during the first three months of the 1933-34 growing season was in marked contrast to the weights obtained during the previous season. The yields increased from a fair figure in November to a maximum (160 grams) in January and then dropped rapidly to the low value of only 50 grams at the conclusion of the growing season in March.

Heavy downpours were experienced in November followed by good average rains in December and January. The rapid falling off in amount of rainfall combined with high temperatures coincided with greatly decreased yields in February and March.

That the prevailing climatic conditions and not the number of missing plants were mainly responsible for low yields during the previous season is borne out by the higher productivity of the same plants, which were further reduced in numbers during the winter of 1933, in the present growing season.

Temperatures are generally high throughout the growing season and undoubtedly exert a modifying influence on yield. During periods of low rainfall, as was experienced during the greater part of 1932-33 season and in February and March, 1934, the high temperatures, through causing high evaporation of soil moisture, would tend to counteract the beneficial influence of rainfall on the production of dry matter.

At the same time it must be borne in mind that different species have different periods of optimum growth activity in the course of the season. The magnitude of a mixed crop during any one month will, therefore, in addition to climate, be influenced by the individual species contributing towards the mixed crop. In this connection it is of interest to note from the data of the 1933-34 season for individual species (c.f. Table III) that while the majority of grasses yielded their highest monthly crops during either December or January, *Cymbopogon plurinoides* and *Themeda triandra* gave optimum yields during November.

## II. TWO-, THREE-, FOUR-, SIX-, AND EIGHT-MONTHLY CUTS.

The average values for the dry matter obtained from the ten species at each cutting have again been chosen as a basis for discussion. Table IV gives the mean composition and weights of the herbage from the different frequencies of cutting for the period February, 1932, to January, 1934, while the detailed data for the ten species over the latter half of the experimental period are presented in Tables B-F of the appendix.

The determination of the reaction of pasture species to varying systems of defoliation and the working out of the most efficient method of treatment with regard to the production of dry matter and essential nutrients are matters of considerable importance to the grazier. On account of the variable climatic conditions of South Africa it is realised that work of this nature will have to be extended over several years before any definite conclusions may be drawn.

It has been demonstrated in the previous paper on this work that when pasture species are allowed to grow to maturity their nutritive value is greatly decreased and that this decrease is further enhanced by allowing the grasses to become dry on the veld. At the same time too frequent defoliations impair the persistency of the grasses and may be expected to result in relatively low yields.

While it is, therefore, necessary for the purpose of optimum production by animals to have pastures containing a sufficiently high content of protein and essential minerals, it is equally important to space the rest periods in such a manner that the pasture may persist in yielding large quantities of nutritious food.

In considering the data from the present investigation it should be borne in mind that the herbage from a two-monthly, three-monthly, etc., cut does not necessarily represent the result of continuous growth during that length of time. As stated under "details of plot experiment" in the preliminary report a definite scheme of cutting at fixed intervals irrespective of the stage of growth was decided upon. This means that the dates on which samples for the different frequencies of cutting had to be taken were definitely fixed at the commencement of the experiment. During winter when practically no growth takes place no samples were, of course, taken. The result of this system is, to quote an example, that the four-monthly cut of September, 1932, was composed of young succulent growth following upon the first rainfall for the season in the second half of the month. Generally speaking, however, the interval between two cuts except during winter and the drought during the latter part of 1933 (until November, 1933) represents the length of time that more or less active growth did take place. These intervals could, of course, also be regarded as rest periods for the grasses between cuttings.

### *Discussion of Results.*

#### *(a) Season 1931-32.*

The data for this season are limited to two two-monthly cuts in March and May, two three-monthly cuts in April and July and a four- and six-monthly cut for May and July, respectively.

The rapid falling off in nutritive value of the herbage if permitted to grow undisturbed from the end of January to maturity and then allowed to remain on the plots is demonstrated by the composition of the dry matter for a two-, three-, four-, and six-monthly cut in March, April, May and July respectively.

The two- and three-monthly cuts in May and July respectively bring out the effect of defoliations in March and April on the composition of the herbage resulting from growth during the respective rest periods. The insignificant amount of rainfall after March coupled with decreasing day and night temperatures as the season advanced to the dry and cold winter months had the effect of greatly reducing the productivity of the grasses. The weights for the dry matter of the two-monthly cuts in March and May were 800.0 and 130.0 grams, respectively, while the yield for the three-monthly sample in July was only 70 grams in comparison with 842.0 grams for the April three-monthly cut.

The smaller yields obtained from the aftermath cuts were in part compensated for by the better quality of the herbage, which was characterised by higher figures for crude protein, phosphate and lime, and lower values for crude fibre.

TABLE IV.

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude protein.	CaO.	K <sub>2</sub> O.	MgO	Na <sub>2</sub> O.	Cl.	Soluble Ash.	Crude Fibre.	Total yield in grams.
TWO MONTHLY CUTTINGS.										
March, 1932.....	27	8 0	40	2 08	34	15	57	—	36 3	800 0
May, 1932.....	31	11 4	76	2 03	60	11	62	—	26 8	130 0
September, 1932.....	39	14 2	69	2 32	51	—	57	—	27 2	72 0
November, 1932.....	26	11 0	72	2 45	63	14	67	—	28 8	256 0
January, 1933.....	26	12 0	62	2 03	55	12	66	—	31 1	406 0
March, 1933.....	32	12 6	68	2 51	68	20	81	—	27 4	168 0
May, 1933.....	24	10 2	79	1 56	72	18	76	4 27	27 5	76 0
November, 1933.....	34	15 9	79	2 34	69	35	51	3 00	30 3	148 0
January, 1934.....	19	7 7	40	1 63	45	22	38	3 33	37 9	490 0
THREE MONTHLY CUTTINGS.										
April, 1932.....	17	5 9	46	1 54	40	13	43	—	34 3	842 0
July, 1932.....	27	10 9	76	1 61	64	07	79	—	26 6	70 0
October, 1932.....	30	12 5	72	2 57	63	14	72	—	27 1	206 0
January, 1933.....	23	11 4	60	2 07	52	10	61	—	30 6	500 0
April, 1933.....	21	9 2	66	1 63	50	—	55	—	27 4	218 0
January, 1934.....	14	6 2	39	1 30	42	15	32	2 81	40 5	1070 0
FOUR-MONTHLY CUTTINGS.										
May, 1932.....	13	5 0	50	1 40	30	097	42	—	34 6	845 0
September, 1932.....	47	16 1	78	2 72	33	11	86	—	25 4	62 0
January, 1933.....	22	10 0	61	1 95	49	078	59	—	30 0	514 0
May, 1933.....	21	9 5	79	1 57	65	11	53	4 10	27 4	158 0
January, 1934.....	14	5 8	41	1 45	41	14	34	2 98	39 4	920 0
SIX-MONTHLY CUTTINGS.										
July, 1932.....	097	3 5	52	1 22	40	055	39	—	35 4	736 0
January, 1933.....	21	9 1	59	1 95	48	070	56	—	29 9	642 0
July, 1933.....	18	8 1	83	1 11	61	085	41	3 48	28 8	110 0
January, 1934.....	15	6 4	40	1 41	42	13	35	2 92	39 8	818 0
EIGHT-MONTHLY CUTTINGS.										
September, 1932.....	12	4 5	58	82	39	045	23	—	35 0	783 0
May, 1933.....	14	5 5	61	1 05	44	049	31	2 99	31 2	745 0
January, 1934.....	17	6 0	40	1 40	43	060	34	2 86	41 8	1250 0



However, for the purpose of estimating the practical value of the different frequencies of cutting the yield and composition of the herbage from the two-monthly cuts in March and May should be compared with the four-monthly cut at the end of the latter month, the yield and composition of the herbage obtained from the three-monthly cuts in April and July should be considered in relation to the six-monthly cut in July, and lastly, the aggregate yield and composition of the crops from any one system with that from any other system.

The relevant data are given in Table V below.

TABLE V.  
(Yields in grams.)

	Two-monthly cuts.	Three-monthly cuts.	Four-monthly cuts.	Six-monthly cuts.
Total dry matter produced.....	930.0	912.0	845.0	736.0
Total crude protein produced....	78.8	57.3	42.2	25.8
Total P <sub>2</sub> O <sub>5</sub> produced.....	2.56	1.62	1.10	0.71

It should be pointed out that growth was most active during February and March, thereafter falling off rapidly in activity to practically no growth after the end of May. The three-, four- and six-monthly hay crops taken at the end of April, May and July, respectively, may therefore be expected to be of a somewhat similar magnitude. The hay cut in July was dry with most of the seeds fallen out, consequently the smaller weight of hay for this harvest could partly be ascribed to losses through exposure to the weather.

There is no significant difference between the aggregate values for dry matter produced by cutting at two- or three-monthly intervals. An increase for any of these two systems over the weights obtained from single harvests in either May or July is, however, indicated.

The production of crude protein and phosphate is significantly higher under the two-monthly system than under any other method of cutting, the amounts of these nutrients decrease steadily as the intervals between cuts become greater.

(b) Season 1932-33.

The collection of samples for this period was commenced at the end of September following upon the first rainfall for the season on the 20th of September. Rainfall was below normal throughout and growth practically ceased at the end of April, the last sample taken in July, a six-monthly cutting, being composed entirely of dry herbage.

The eight-monthly cut taken in September, 1932, was largely composed of old growth from the previous season and will not be considered in discussing the results for the present season. The harvest eight months afterwards, in May, 1933, may be taken to represent undisturbed growth during the whole of the 1932-33 season.

In view of the fact that growth ceased at the end of April the aggregate yields of dry matter, crude protein and phosphate obtained under the different frequencies of cutting from September, 1932, to July, 1933, are comparable. For the purpose of bringing out the relative merits of the various systems more clearly the data are given in Table VI below.

TABLE VI.  
(Yields in grams.)

	Two-monthly cuts.	Three-monthly cuts.	Four-monthly cuts.	Six-monthly cuts.	Eight-monthly cuts.
Total dry matter produced....	978· 0	924· 0	734· 0	752· 0	745· 0
Total crude protein produced..	115· 8	102· 7	76· 4	67· 3	41· 0
Total P <sub>2</sub> O <sub>5</sub> produced.....	2· 73	2· 23	1· 75	1· 55	1· 04

Cutting at two-monthly intervals was initiated during the awakening period of the plants in September and the herbage for this first cut was entirely composed of young leafy growth.

The cuts for November and January both reached the hay stage (c.f. Table C in appendix of previous paper) in that all the plants were in full flower, the yield for the latter month being the highest for a single cut under this system for the season, following upon the best rainfall for the growing period during the previous month.

Two further harvests in March and May were obtained, the herbage for March was almost entirely in the flowering stage of growth, while in that obtained in May short leafy growth, affected by frost, predominated. These two aftermaths made considerable contributions to the bulk of hay obtained in November and January.

The majority of grasses were in the flowering stage when the first cut under the three-monthly system was taken in October. The longer rest period during the ensuing three months when growth was most active for the season was responsible for a greater yield in January than was obtained at the same date from a two-monthly cut. At the same time the herbage was more mature, the seeds of fully half of the ten species responsible for the yields were beginning to fall out.

There was only one relatively small aftermath cut at the end of April composed partly of dry herbage in varying degrees of maturity.

It would appear that a moderate hay crop at the flowering stage might be more favourable to aftermath production than a heavy hay crop at the post-flowering stage taken at the same date. In view of the fact that two cuts were taken after the two-monthly cut in January as against only one subsequent to the three-monthly cut of the same date, such a conclusion must be regarded tentatively but is nevertheless borne out by the work of Stapledon (1924). Metereological conditions and the growth habits of the individual species composing a mixed pasture are other factors which may influence the results.

Following upon the commencement of the four-monthly system with a pasture cut, that is, in the pre-flowering stage, in September, a late hay crop was harvested four months afterwards, i.e. at the end of January. Although the actual weight of hay obtained from this cut is not greatly in excess of the weight from the three-monthly crop of the same date the smaller aftermath crop in May tends to add weight to the tentative conclusion drawn above in regard to the influence of a heavy hay crop on the production of aftermath.

Working with drills of cocksfoot and swards Stapledon (*loc. cit.*) concluded that the yield of both hay and aftermath were influenced to a considerable extent by the date of "putting up" to hay, that is to say the date on which the herbage was cut for the last time before the hay crop. In this connection it is interesting to compare the aggregate yields obtained from the two hay crops under the two-monthly system in November and January and the weights of the single hay crops from the three-, four-, and six-monthly cuts at the latter date, when the dates for the first cuts in each case are regarded as "putting up" dates and the weights of such first cuts not included. The relevant data as well as the total aftermath production following the hay crops under the different systems in January are given in tabular form below.

TABLE VI (*b*).  
(Hay and Aftermath in Grams.)

	Two-monthly system.	Three-monthly system.	Four-monthly system.	Six-monthly system.
"Putting up" date.....	27/9/32	27/10/32	27/9/32	27/7/32
Weight of hay.....	662.0	500.0	514.0	642.0
Weight of aftermath.....	244.0	218.0	158.0	110.0

The evidence suggests, firstly, that two hay crops during the most productive part of the season (November to January) result in a higher aggregate yield than is obtainable from a single harvest, secondly, that a single crop from plants "put up" to hay during the dead period (July) is responsible for a greater bulk of hay than a single crop resulting from plants cut for the last time during the awakening period (September or October), and, lastly, that heavy hay and relatively heavy aftermath crops do not occur during the same season, while two aftermath crops after a "flowering stage" hay cut outyield a single aftermath cut following upon a "waning flowering stage" hay crop.

From a consideration of the data given in Table VI it may again be concluded that, while there is no significant difference in the production of dry matter during the entire growing season by the two- and three-monthly systems, and the gross yield of dry matter is decreased by lengthening the intervals between cuts to four, six or eight months, the system of cutting at two-monthly intervals appears to be the most efficient in respect of the production of crude protein and phosphoric acid.

As stated previously the eight-monthly cut taken in May represents undisturbed growth for the entire growing season. The hay was described as "mainly brown, seeds falling out" and weighed approximately the same as the aggregate yields from the four- or six-monthly cuts. The percentages phosphate and crude protein of the hay from this single harvest were the lowest for any of the cuts taken during the season. A system of reserving certain areas during the growing season for winter pasture is still extensively practised in some parts of the country and apart from poorer production of dry matter indicated for such a system by the present investigation the phosphate and crude protein content of this type of grazing is quite insufficient for even poor milk production under ranching conditions where no supplementary feeding takes place.

Compared with the practice referred to above the system of pasture management suggested by the results of cutting at two-monthly intervals indicates the direction in which the potentialities of our grasslands could with advantage be explored. Moderately good figures for both crude protein and phosphorus were obtained for all the two-monthly cuts, depending largely on the degree of maturity reached by the herbage of the respective crops. Thus, the pasture cut with which the system was initiated in September gave higher values for  $P_2O_5$  and protein than any of the subsequent harvests. All other mineral constituents remained high throughout, while the figures for crude fibre were not much higher than were obtained in the herbage from a more severe system of cutting, previously discussed.

The composition of the herbage from the first cuts of the three- and four-monthly systems in October and September respectively, is comparable with that obtained from a monthly cut while the January yield from these two systems contains slightly less crude protein and phosphoric acid than the herbage obtained at the same date under the two-monthly system. The values for these constituents in the aftermaths of the three- and four-monthly systems vary similarly from the figures obtained in the aftermaths of the two-monthly system. Lime values are throughout higher and potash figures generally lower in the aftermath cuts under all systems compared with the values for these constituents in the respective hay crops. All aftermath harvests are characterised by relatively low values for crude fibre.

Judging from the composition of the six-monthly cut in January the hay is slightly less nutritious than that obtained under the four-monthly system at the same date, while its aftermath harvested in July shows the poorest composition compared with any crop under all systems for the entire growing season, with the exception of the eight-monthly cut in May.

#### (c) *Season 1933-1934.*

The data for this season are limited to a pasture cut under the two-monthly system in November and hay crops for all the systems in January. As previously stated the rainfall for November was exceptionally high and was well distributed over the month. The light showers registered before this date had practically no effect on growth.

The pasture cut taken at the end of November from the second rows of the plots was composed entirely of young succulent growth, the composition of the dry matter was very much the same as that from the monthly cuts taken on the same date, and need not be discussed here.

Of greater interest is the effect of this pre-cut in November on the average yield of hay at the end of January in relation to the mean weights of the hay crops obtained from the third, fourth, sixth and eighth rows, which had been cut for the last time in April, May, July and May of the previous season, respectively. The average yields obtained in January from the respective rows of the ten species as well as the dates on which the plants were cut for the last time before the hay crops are given in Table VII below.

TABLE VII.  
(Yields in Grams.)

	Two-monthly system.	Three-monthly system.	Four-monthly system.	Six-monthly system.	Eight-monthly system.
Cut for the last time on....	27/11/33	27/4/33	27/5/33	27/7/33	27/5/33
Weight of hay in January...	490.0	1,070.0	920 0	818 0	1250.0

No data in regard to growth after January for the systems other than monthly cuts are available. The pronounced influence, however, of the date of "putting up" to hay on the hay crops at the end of January is again apparent from the data in Table VII. Generally speaking the plants cut for the last time towards or after the termination of the previous growing season produced approximately twice as much hay as plants cut for the last time at the pre-flowering stage in November of the present season.

It is difficult at this stage of the investigation to explain the variations in yield of the series of plants "put up" to hay at the conclusion of the previous season. Whether cutting for the last time in April, May or July will influence the hay crops at a specific date during the following season is a matter which will be investigated in the near future when it is hoped to have bigger plots and more definite conclusions in regard to factors influencing yield may be drawn.

Compared with previous seasons the hay crops obtained in January are relatively heavier; this is attributed to the favourable meteorological conditions obtaining since November.

A consideration of the chemical data given in Table IV reveals the fact that as a result of the favourable climatic conditions mentioned above the composition of the herbage cut at the end of January, that is after only three months undisturbed growth, is comparable with the poor quality hay obtained in May of the previous season after eight months undisturbed growth. Apart from the low

values for the more essential constituents, phosphoric acid and crude protein, all other minerals determined, with the exception of sodium, show greatly decreased values as against markedly increased values for crude fibre.

The analysis for the two-monthly hay crop shows slightly better figures than that for the other systems. Nevertheless the evidence seems to suggest that during the "zenith period" of growth of seasons with high rainfalls the intervals between successive cuttings will have to be reduced to something less than two months for the production of herbage of greatest feeding value. Earlier in this paper mention was made of the fact that comparatively low values were obtained even in the dry matter of the herbage resulting from cutting at monthly intervals at this period of the 1933-34 growing season. On the other hand, from evidence to be considered presently, a more drastic system of cutting will have a depressing effect on the yield of herbage. The data are as yet too scanty to decide upon a definite system but it is apparent that a golden mean between quantity and quality of herbage in relation to meteorological conditions will have to be found if the maximum feeding value of our grasses is to be obtained.

### III. THE EFFECT OF CUTTING AT MONTHLY AND TWO-MONTHLY INTERVALS ON THE AGGREGATE YIELD OF DRY MATTER, CRUDE PROTEIN AND PHOSPHORIC ACID.

With the exception of the 1933-34 season when climatic conditions were above normal and the data incomplete it has been demonstrated that the highest aggregate yields of dry matter, crude protein and phosphate have been obtained by harvesting at two-monthly intervals. It will be of interest to compare these results with those obtained by cutting at monthly intervals over the same period. The data for the monthly cuts in February and March, 1934, are not included. The average aggregate yields of dry matter, crude protein, and phosphate, obtained by cutting the respective portions of the ten plots of grasses at monthly and two-monthly intervals for each of the three seasons concerned are given in Table VIII below.

TABLE VIII.  
(Yields in Grams.)

	Monthly system.			Two-monthly system.		
	Dry matter.	P <sub>2</sub> O <sub>5</sub> .	Crude protein.	Dry matter.	P <sub>2</sub> O <sub>5</sub> .	Crude protein.
Season 1931-32...	590.0	2.50	74.8	930.0	2.56	78.8
Season 1932-33...	430.0	1.43	62.0	978.0	2.73	115.8
Season 1933-34...	436.0	1.28	54.0	638.0	1.43	61.3

In considering these results it should be remembered that the aggregate yields from each of the three seasons are not comparable one with the other, the data for the 1931-32 season relate to only the latter half of the season since the investigation was initiated in February, 1932, while for the last season data are given only up to the end of January, 1934. It is to be noted, however, that the whole of the dry 1932-33 season yielded but little more dry matter under the two-monthly system and a fair amount less under the monthly system than was obtained from a portion of the 1931-32 season under the two systems respectively.

The outstanding feature of the data in Table VIII is the greatly increased aggregate yields of dry matter obtainable from the more lenient system of cutting. This disparity becomes more exaggerated in proportion as the climatic conditions are unfavourable during the growing season as is apparent from the data for the 1932-33 season.

While cutting at monthly intervals produces herbage of higher feeding value during seasons of favourable meteorological conditions, the shortened interval does not appear to improve the quality of the herbage appreciably during dry spells. During the 1932-33 season the production of dry matter was more than doubled by a system of cutting at two-monthly intervals and at the same time the aggregate yields of crude protein and phosphoric acid were almost twice that produced by cutting at monthly intervals. At the same time the evidence seems to suggest that with more favourable growth conditions a smaller yield under the more drastic system of cutting might be compensated for by the better quality herbage, provided, of course, the grasses are able to stand the more drastic treatment successfully. In this connection the insignificant differences in aggregate yields of crude protein and phosphoric acid obtained for the two systems for the portion of the 1931-1932 season are worthy of note.

No data are available in respect of the effect of frequency of cutting on the development of the root systems of the plants although it was observed that the percentage of plants dying as a result of the various treatments was greatest in the portions of the plots cut at monthly intervals. This fact together with the depressing effect of drastic systems of cutting on the yield of succeeding seasons as pointed out by Stapledon (*loc. cit.*) may have been responsible for smaller yields under the monthly system than under the two-monthly system of cutting during the two seasons following upon the initiation of the investigation in February, 1932, but does not, on the other hand, explain yield in favour of the two-monthly system as indicated by the data for the first season of the experiment, when all the plants could be taken to have been in their pre-experimental condition.

It may be concluded that during dry seasons cutting at two-monthly intervals is definitely preferable to any of the systems studied in this investigation, while during periods of abnormal rainfall the most efficient system appears to be to cut the herbage at a date somewhere between one and two months depending upon the growth habits and resistance of the individual species composing the mixed pasture.

## SECTION 2.—NEWLY ESTABLISHED SPECIES.

As already stated, in addition to the investigation reported on in Section one of this report work was started on eleven other species of grasses towards the end of the 1932-33 growing season. The grasses on all these plots were cut on the 28th February and the same system of taking samples for analysis, described in the previous publication, initiated on the 28th March. Owing to the lateness of the season when cutting was started few cuts were obtained for the monthly samples and growth was generally poor. No attempt will therefore be made to discuss the results for the portion of the 1932-33 season in detail, i.e. March-April, 1933.

Table G in the appendix gives the dates of cutting, the yield of dry matter and the percentage composition together with a short description of the stages of growth of the monthly cuts obtained from March, 1933, to March, 1934, while similar data for cuts obtained up to the end of February, 1934, under all other frequencies of cutting are presented in Tables H-M of the appendix.

### DISCUSSION OF RESULTS.

#### I. MONTHLY CUTS.

Reference to Table G in the appendix reveals the following: Growth ceased after the April (1933) cuts in the case of five species while the remaining six species yielded the last but relatively small crops for the season at the end of May. In spite of the fact that the first good rains for the 1933-34 season fell in November two species *Chrysopogon montana* and *Elyonurus argenteus* began yielding small crops of herbage in August. It was possible to take monthly samples from *Alloteropsis semialata* from the end of September, while *Antheperu pubescens* and *Eragrostis plana* yielded the first samples under this system of cutting for the season in October. Samples of herbage were also obtained from the *Eragrostis* sp. and *Paspalum dilatatum* plots from the end of August, but growth on these plots cannot be considered to have taken place as a result of the prevailing climatic conditions alone, since frequent watering of adjacent plots where new species were being established during August might have exerted its influence on growth on the adjacent sides of the *Paspalum* and *Eragrostis* plots. Following the first heavy rains for the season in November growth proceeded actively on all the eleven plots concerned.

The average percentage composition of the dry matter of the monthly cuts of the eleven species is given in Table IX below, the mean yield being included in the table. It is proposed to discuss briefly the results for the 1933-34 season only, those for March, April, and May, 1933, being left out for the reason already stated.

The dry matter of the composite sample for September, composed almost entirely of short leafy growth, is slightly richer in crude protein and phosphate and gives a lower figure for crude fibre than any of the composite samples collected during the season. This peak for phosphate in early spring is in agreement with the findings of Henrici (1928 and 1930). The data for September herbage samples



TABLE IX.

Date of Cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.
March, 1933.....	.43	14.0	.52	2.24	.46	.13	.78	—	32.0	154.0
April, 1933.....	.39	12.8	.54	2.03	.44	.14	.72	—	30.8	130.0
May, 1933.....	.41	14.0	.57	1.79	.43	.23	.71	4.04	26.9	37.0
September, 1933.....	.47	14.7	.56	2.30	.52	—	.57	4.33	27.4	68.0
October, 1933.....	.34	13.0	.54	1.77	.49	—	.52	4.12	31.6	62.0
November, 1933.....	.30	13.0	.50	2.18	.46	.20	.44	4.04	34.8	230.0
December, 1933.....	.33	13.3	.47	2.07	.42	.17	.44	3.83	34.1	130.0
January, 1934.....	.25	11.0	.39	1.89	.37	.14	.44	3.57	36.4	220.0
February, 1934.....	.32	11.7	.47	1.89	.40	.12	.45	3.99	34.6	150.0
March, 1934.....	.35	11.9	.52	1.92	.41	—	—	3.88	34.1	110.0

TABLE X.

(PERIOD OF GROWTH 1 month, 2 months, etc., up to 12 months.)

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.
March, 1933.....	.43	14.0	.52	2.24	.46	.13	.78	—	32.0
April, 1933.....	.29	10.3	.59	1.69	.47	.14	.71	—	31.0
May, 1933.....	.19	7.8	.66	1.26	.51	.12	.64	3.34	30.4
June, 1933.....	.14	5.9	.71	.91	.52	.12	.49	3.04	31.4
July, 1933.....	.11	5.4	.75	.87	.53	.14	.47	3.11	31.5
August, 1933.....	.12	5.5	.76	.86	.50	.12	.48	2.96	32.7
September, 1933.....	.14	6.2	.62	.63	.35	.08	.23	2.31	32.9
October, 1933.....	.11	6.0	.57	.56	.29	.07	.21	2.50	32.1
November, 1933.....	.22	9.7	.49	1.74	.38	.11	.34	3.51	36.9
December, 1933.....	.18	6.7	.41	1.57	.36	.10	.35	3.03	39.4
January, 1934.....	.14	5.6	.33	1.37	.29	.08	.28	2.61	39.4
February, 1934.....	.13	5.6	.35	1.25	.32	.08	.25	2.69	40.0

in the present investigation are, however, too scanty for a definite conclusion. The species composing the mixed sample and the climatic conditions are factors which will no doubt influence the variations in phosphate content in the course of the growing season. There is, for instance, no appreciable difference in the  $P_2O_5$  values of *Chrysopogon Montana* and *Eragrostis* sp. for September and March (c.f. Table G). A similar phenomenon was noted in the case of some of the older established species during the 1932-33 season (c.f. Table IV of previous paper).

Generally speaking, the average percentage composition of the monthly cuts of the eleven species vary in a manner similar to that of the other ten species, discussed in Section one of this paper, from November to March. Minimum values for all the inorganic constituents and crude protein and a maximum for crude fibre were again obtained in the composite sample for January. Phosphate and crude protein display parallel variations, while the latter constituent and crude fibre fluctuate in opposite direction from October to March.

It is difficult at this stage of the investigation to decide upon the best grasses among the species studied. *Alloteropsis scmiulatas* certainly gave the highest figures for phosphate and crude protein from month to month throughout the season, but yielded, on the other hand, a smaller aggregate weight of herbage for the period from September to March than any of the other species. *Ischaemum glaucostachyum* was the poorest in respect of phosphate and crude protein and was responsible for a relatively small aggregate yield of dry matter. *Chrysopogon montana*, *Eragrostis* sp., *Paspalum dilatatum* and *Elyonurus argenteus* appear to have stood up best to the dry weather conditions experienced from May to October, 1933, the last-mentioned grass showing green shoots even during the winter months.

*Paspalum* gave the highest aggregate yield of dry matter for the period September to March. *Eragrostis plana* and *Eragrostis* sp. were also high producers of dry matter under the system of cutting at monthly intervals. While yielding herbage of relatively high nutritive value the frequent defoliations seems to have impaired the yielding capacity of *Chloris gayana*.

## II. SUCCESSIVE MONTHLY CUTS.

For the purpose of comparison with the data for the older established species reported on in the previous paper (1934) the average percentage composition of the eleven species for the period March, 1933, to February 1934, is presented in Table X at the following periods of growth: 1 month, 2 months, 3 months, and so on up to 12 months.

A rapid drop in the percentage phosphate and crude protein as the grasses mature is again in evidence, the herbage cut in July containing only about 25 per cent. of the phosphorus and 38 per cent. of the crude protein present in the crops for March. After a light shower in the beginning of September a slight improvement is noticeable for the samples taken at the end of the month. This increase in percentage

phosphate and crude protein disappears during almost two months of dry weather with the result that the percentage composition of the herbage cut at the end of October is similar to that of the samples harvested at the end of July. Contrary to the findings of the previous year a remarkable improvement in the percentage composition of the herbage occurs in the samples for November. Two factors, viz., excessive loss of mature herbage, low in phosphate and crude protein, due to strong winds during the dry weather of the greater part of September and October, and relatively large amounts of new succulent growth following upon the copious rains during November may be said to have been responsible for the greatly increased phosphate and crude protein values for November. Reference to the descriptions of the samples from individual species given in Table M of the appendix shows that old growth from the previous season must have disappeared completely from some of the plots during December or January. Conditions for new growth remain favourable to the end of January, 1934, but owing to the increasingly higher degree of maturity obtained by the herbage composing the samples for 10-, 11-, and 12-monthly cuts at the end of December, January, and February, respectively, the percentage phosphate and crude protein decrease gradually from November to February.

The percentage of potash and chlorine show a persistent drop from March to October. It is to be noted that no improvement occurs as a result of new growth after the light shower in the beginning of September, a finding similar to the results of the previous season (du Toit, *et al.*, 1934). A striking improvement, especially in the percentage of potash is, however, brought about by the heavy rains of November. It has been suggested in the discussion of the potash figures in the previous paper that the old growth forming part of the mixed sample of herbage after new growth has commenced following upon the first spring rains contained a lower percentage of  $K_2O$  than the same old growth did prior to the rains. For the purpose of elucidating this point a number of mixed samples cut at the end of November have been divided into old and new growth and analysed separately. Table XI below gives the percentage composition of the October samples, composed almost entirely of growth from the previous season, and of the old and new growth portions of the samples cut at the end of November for two of the species studied. The analysis of the mixed samples for the latter month are included in the table.

A study of the results given in Table XI and reference to the data in Table X reveal the fact that the process by which minerals and protein are lost from the herbage portions of plants commences after approximately two months growth when the plants are fully mature, probably earlier (c.f. Richardson, *et al.*, 1931, 1932), continues to at least November and appears to be aggravated by heavy rains as experienced during this month. The actual mechanism of the process has not been studied. In addition to the work of Richardson referred to above the finding of Henrici (1930) that re-migration of  $P_2O_5$  from leaves and haulms to root and root neck takes place during autumn is of interest in this respect, while it seems quite plausible that rain may have a leaching effect on the constituents contained in old dry grasses which have been subjected to adverse

metereological conditions, such as frost during winter and the subsequent weathering of the dead aerial parts. Van Wyk (1933) believes that rain probably has a leaching effect on the constituents of grasses even at an active stage of growth. The fact that a large quantity of potash remains in a soluble form throughout the growth of the plant as demonstrated by Bartholomew and Janssen (1929) will probably facilitate leaching out of this constituent, and more so in old dead grass of the previous season's growth.

TABLE XI.

*Anthephora pubescens.*

Date of cut and description of growth.	P <sub>2</sub> O <sub>5</sub> .	Crude Pro- tein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.
October, 1933.							
Old growth.....	·09	6·1	·95	53	·50	·021	18
November, 1933.							
Old growth.....	·04	2·7	·56	·26	·43	·020	·06
New growth.....	·27	10·6	·43	2 35	1·19	·055	·71
Mixed growth.....	·22	8·8	·54	1·85	·97	·037	·56

*Paspalum dilatatum.*

October, 1933.							
Old growth.....	·07	4·7	·58	·65	·32	·027	·08
November, 1933.							
Old growth.....	·05	3·4	·59	23	·24	·020	·04
New growth.....	·37	14·2	·52	3 53	·48	·077	·48
Mixed growth.....	·22	10·7	·53	2·08	·41	·053	·26

However, from what has been said it is apparent that the proportion of new to old growth in the mixed samples cut after the first spring rains will largely determine the mineral composition of such mixed samples. A glance at Table A in the appendix of the previous paper shows that during October, 1932, the samples were composed mainly of dry mature herbage from the previous season while in the November (1932) samples old and new growth were present in approximately equal quantities, with the result that no improvement in the percentage composition of the samples, in spite of new growth, was found.

On the other hand, new growth predominated in the samples for November, 1933 (c.f. Table M in appendix) following upon climatic conditions which caused the removal of large quantities of old growth from the plots during October and favoured rapid new growth during November with the result already indicated.

From November to February potash and to a lesser extent chlorine values in Table X drop in a manner similar to phosphate and crude protein.

The lime, magnesia and soda values in Table X, while showing appreciable variations, do not appear to be influenced to any marked degree by the stage of maturity of the herbage. Lime and magnesia tend to increase from March to July, thereafter showing decreased values, especially during the months December and January when growth was most active. In this connection the findings of Richardson and associates (*loc. cit.*) are of interest. According to these workers calcium, unlike phosphorus, potash and nitrogen, is absorbed continuously throughout growth and the percentage intake tends to increase with the development of the plant. Furthermore, the suggestion of Maskell and Mason (quoted by Richardson) that the calcium, once in the cells of the plant is either precipitated or combined with tissue in such a way that little remains in solution would explain the consistency high values obtained for calcium in the samples from March to July as against greatly decreased values for phosphorus protein and potassium.

Sodium values which are remarkably low for all species except for the outstandingly high figures for *Chloris gayana*, show very little variation from March to August. Values decrease during September and October but improve again after good rains in November followed by a second drop in January and February.

Figures for crude fibre in Table X remain practically constant from March to September. A gradual rise to a higher level, which is maintained for the rest of the period under discussion, in December is indicated.

The general conclusions drawn from the data of the eleven species reported on in the previous paper apply equally well to the data for the species of grasses under discussion. Briefly, phosphorus and crude protein values are deplorably low as the period of growth advances beyond two months. Sodium values are generally low and the possibility of being present in inadequate amounts for production in certain pastures is suggested, while all other constituents determined appear to be present in abundance.

### III. THE INFLUENCE OF FREQUENCY OF CUTTING ON THE PRODUCTION OF DRY MATTER, PHOSPHATE AND CRUDE PROTEIN.

For this purpose the data for the 1933-34 season alone will be considered. The six-monthly cuts taken at the end of February, 1934, represent undisturbed growth from the end of August and are comparable with the aggregate yields obtained from the one-, two-, three-, and four-monthly systems and resulting from growth during the same period of six months. The average percentage composition and yield of herbage obtained under the monthly system of cutting from the species studied are to be found in Table IX earlier in this paper while similar data relating to the other frequencies of cutting are given in Table XII.

TABLE XII.  
*Two-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble Ash.	Crude fibre.	Total yield in grams.
October, 1933.....	.28	11.2	64	1.82	.53	—	.53	4.26	28.5	71.0
December, 1933.....	.22	8.9	.40	1.78	.37	.16	.43	3.33	37.9	636.0
February, 1934.....	.20	8.0	.37	1.54	.34	.12	.44	3.18	39.5	640.0
<i>Three-monthly Cuttings.</i>										
November, 1933.....	.26	11.8	.51	2.14	.44	.18	.46	4.01	36.1	289.0
February, 1934.....	.15	6.9	.33	1.36	.34	.10	.37	2.80	38.8	535.0
<i>Four-monthly Cuttings.</i>										
October, 1933.....	.26	11.0	.73	1.87	.57	—	.68	4.27	31.4	57.0
February, 1934.....	.13	5.1	.34	1.20	.32	.07	.31	2.63	40.1	1282.0
<i>Six-monthly Cuttings.</i>										
February, 1934.....	.12	5.4	.34	1.17	.32	.06	.30	2.62	39.8	1125.0

From the data in Tables IX and XII the aggregate production of dry matter, phosphate and crude protein for the six months September to February under all treatments have been calculated and are presented in tabular form below.

TABLE XIII.  
(Yield in Grams.)

	Monthly cuts.	Two-monthly cuts.	Three-monthly cuts.	Four-monthly cuts.	Six-monthly cuts.
Dry matter.....	860.0	1347.0	824.0	1339.0	1125.0
P <sub>2</sub> O <sub>5</sub> .....	2.68	2.88	1.55	1.80	1.35
Crude protein.....	107.0	115.7	71.0	71.7	60.7

In respect of dry matter produced there is no difference between the two- and four-monthly systems, while the single harvest in February shows a slightly lower yield than was obtained under either of these treatments. The aggregate yields of dry matter under the monthly and three-monthly systems are of a somewhat similar magnitude but are only about 60 per cent. of the weights obtained under the two- or four-monthly systems. The low yield under the three-monthly system affords additional evidence with regard to the influence of the date of "putting up" to hay on the subsequent production of dry matter.

Reference to Table XII shows that this system was initiated in November after the first good rains for the season. Growth actually commenced before this date but became active only during November. The effect of a defoliation during this month on the production of hay in January under a two-monthly system has been demonstrated by the results given in Table VII (see Section 1). In the case under consideration the hay crop was harvested a month later, in February, but was nevertheless influenced in a similar manner by the late pre-cut of November.

While, as stated above, the yield of dry matter under a two-monthly system does not exceed that obtainable under a four-monthly system the gross production of the essential nutrients phosphate and protein under the former system of cutting is greater than under any of the more lenient treatments, a finding in agreement with the conclusions drawn from the data of previous seasons discussed earlier in this paper. The possibility that a smaller yield will be compensated for by a better quality of the herbage obtained from a more drastic system of cutting during periods of favourable climatic conditions is again suggested by the insignificant difference in the gross yields of phosphate and protein under the monthly and two-monthly systems of cutting (c.f. Table XII).

#### GENERAL CONCLUSIONS AND SUMMARY.

The investigation which has been dealt with in this paper is a continuation of earlier work carried out in 1932 on species of grasses grown on separate plots and exposed to the same climatic conditions

while being subjected to the following system of cutting: A portion of each plot was cut at monthly intervals, another portion of the plot was cut at two-monthly intervals, a third portion at three-monthly intervals, and so on, up to twelve months, when a sample of twelve months' growth was taken off each plot. The grass resulting from each cutting was weighed when air-dry and sampled for analytical purposes. The constituents determined were crude protein, crude fibre, phosphorus, calcium, potassium, magnesium, sodium, and chlorine, while the determination of soluble or silica-free ash was included towards the end of the investigation.

Detailed data relating to all the cuts obtained from individual species from February, 1933, to February, 1934, are tabulated in an appendix. Average values for all the species combined have been chosen as a basis for discussion.

(1) Grasses cut at monthly intervals are highest in percentage phosphorus and crude protein and lowest in percentage crude fibre. With decreased frequency of cutting the crude fibre content rises to a maximum at maturity, while the phosphorus and crude protein contents drop markedly to minima in the old mature herbage cut prior to the commencement of new growth in the following season.

(2) The different frequencies of cutting do not appreciably influence the lime and magnesia contents of the herbage.

(3) Pure species grown on the same soil and exposed to the same climatic conditions show appreciable differences in mineral and protein content when harvested after definite intervals, e.g. one month.

(4) Fluctuations in the chemical composition of species cut at, say, monthly intervals during the growing season are indicated. These variations are attributed mainly to the stage of growth attained by the species and to the rainfall in so far as it influences the stage of growth.

(5) Rainfall is the most important factor governing the yield from monthly cuts.

(6) During dry seasons the phosphorus content of herbage cut at monthly intervals tends to be low. At the same time the percentage lime is high. The crude protein content does not appear to be adversely affected by drought.

(7) The percentage of mineral constituents and crude protein are low and crude fibre high during seasons of plentiful rains.

(8) Cutting at monthly intervals impairs the persistency of most species, *Pennisetum ciliare* and *Cynodon dactylon* being the only grasses not visibly affected by the severe treatment.

(9) The highest yield of dry matter is obtained from pasture species by cutting at two-monthly intervals during the growing season. The evidence suggests that while the yield from fewer cuts or a single harvest may not be much smaller the herbage will definitely be of lower nutritive value.

(10) The aggregate production of crude protein and phosphate is highest by cutting at two-monthly intervals.



(11) The difference in nutritive value between herbage cut at monthly and two-monthly intervals becomes insignificant during seasons of dry weather conditions.

(12) The date on which a system of cutting is initiated is found to exercise a determining influence on the productiveness of herbage plants in the course of the growing season. The production of dry matter seems to be depressed by a first cut during the period when active growth commences.

(13) The practical suggestion which is a corollary to this investigation is that an attempt should be made to prevent natural pastures from growing undisturbed during the entire season if economic utilization of such pasture is to be made. Provided the effects of periodic cutting are at all comparable with grazing by animals this object appears to be attainable by following a system of management in which the pasture is grazed down at least once every two months during the active growing season depending, of course, on the prevailing climatic conditions and the botanical composition of the pasture.

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APPENDIX.  
TABLE A.

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>HYPARHÆXIA HIRTA</b>											
February, 1933.	26	11.3	52	1.64	45	0.18	46	—	—	25.0	Green, short, few flowerheads.
March, 1933.	29	11.4	57	1.95	49	0.16	56	—	28.7	50.0	Green, few flowerheads.
April, 1933.	26	11.4	82	1.98	47	0.18	54	—	28.3	45.0	Green, few flowerheads.
November, 1933.	28	15.1	82	1.23	46	0.34	—	3.76	28.9	44.0	Green, short.
December, 1933.	20	10.6	70	1.23	46	0.34	—	2.70	36.5	100.0	Green, with flowerheads.
January, 1934.	21	8.1	52	1.38	50	0.38	17	3.36	32.8	80.0	Green, short.
February, 1934.	24	11.2	44	1.65	47	0.24	26	3.03	34.8	28.0	Green, short.
March, 1934.	23	9.2	60	1.64	48	Trace	—	—	—	—	Green, short.
<b>THEMIDA TRIANDRA.</b>											
February, 1933.	27	11.1	79	1.14	41	NH	40	—	26.3	20.0	Green, short.
March, 1933.	37	12.9	81	1.35	46	0.18	45	—	27.1	25.0	Green, short.
April, 1933.	32	12.2	74	1.56	42	0.22	51	—	27.1	25.0	Green, short.
November, 1933.	27	12.2	81	1.45	37	0.22	39	3.10	36.7	280.0	Green, few flowerheads.
December, 1933.	25	9.5	62	1.45	40	0.33	33	2.51	36.0	120.0	Green, few flowerheads.
January, 1934.	20	8.3	60	1.19	39	0.33	37	3.01	37.2	96.0	Green, short.
February, 1934.	43	8.6	76	1.90	40	0.33	—	—	—	20.0	Green, short.
March, 1934.	—	—	—	—	39	0.31	—	—	—	—	Practically no growth.
<b>CYMBOPOGON PLYRINOIDES.</b>											
February, 1933.	33	10.1	52	2.24	42	0.52	41	—	—	17.0	Green, short.
March, 1933.	42	12.8	47	2.64	40	0.67	55	—	—	25.0	Green, short.
April, 1933.	42	12.2	—	—	—	—	—	—	—	—	Green, short.
May, 1933.	32	10.5	50	1.84	44	0.82	46	3.30	33.7	15.0	Green, few flowerheads.
December, 1933.	22	10.6	53	1.00	49	0.48	46	3.65	34.1	330.0	Green, few flowerheads.
January, 1934.	20	10.8	53	1.55	43	0.23	52	3.18	29.6	80.0	Green, few flowerheads.
February, 1934.	29	11.6	45	1.45	43	0.62	48	3.28	31.4	52.0	Green, short.
March, 1934.	28	11.8	47	1.67	43	Trace	—	3.26	—	9.0	Green, short.
<b>CYNODON DACTYLON.</b>											
February, 1933.	22	11.1	60	1.22	43	Trace	26	—	27.6	35.0	Green, short.
March, 1933.	25	12.6	60	1.52	33	—	—	—	26.2	35.0	Green, with flowerheads.
April, 1933.	18	10.2	68	1.00	37	0.20	32	—	27.7	35.0	Green, short.
November, 1933.	28	14.1	64	1.76	42	0.63	28	4.06	27.0	146.0	Green, short.
December, 1933.	73	14.1	58	1.91	43	0.67	20	3.83	29.1	42.0	Green, short.
January, 1934.	23	12.6	53	1.61	31	0.44	20	3.50	33.8	352.0	Green, with flowerheads.
February, 1934.	32	12.6	56	1.80	41	0.48	26	4.09	32.5	188.0	Green, short.
March, 1934.	—	—	—	—	—	—	—	—	—	—	Practically no growth.
<b>AMPHILOPHIS INSULATA.</b>											
February, 1933.	24	10.4	60	2.50	46	Trace	53	—	29.8	85.0	Green, few flowerheads.
March, 1933.	32	12.0	62	2.30	46	0.26	74	—	30.1	75.0	Green, few flowerheads.
April, 1933.	28	12.7	60	2.37	48	0.33	53	—	29.8	55.0	Green, few flowerheads.
November, 1933.	27	13.5	83	1.87	71	0.37	52	3.96	29.0	60.0	Green, short.
December, 1933.	28	19.3	66	2.04	57	0.37	43	3.68	38.3	200.0	Green, few flowerheads.
January, 1934.	31	10.4	63	2.09	51	0.25	54	3.85	34.0	104.0	Green, few flowerheads.
February, 1934.	24	11.7	64	1.90	44	0.23	51	4.04	34.5	50.0	Green, few flowerheads.
March, 1934.	25	10.1	58	1.87	50	Trace	—	3.69	—	10.0	Green, short.

TABLE A. (continued).

Date of cutting.	P.O.	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>PERISTETUM CILIARE.</b>											
February, 1933.....	.31	13.2	.52	2.81	.88	.068	.87	—	31.0	100.0	Green, few flowerheads.
March, 1933.....	.36	14.8	.50	3.93	.86	.075	1.04	—	29.7	135.0	Green, with flowerheads.
April, 1933.....	.32	14.6	.51	2.72	.89	.086	.94	—	26.4	85.0	Green, with flowerheads.
November, 1933.....	.38	15.1	.50	4.24	.77	.065	.29	6.86	28.3	247.0	Green, few flowerheads.
December, 1933.....	.39	18.4	.44	3.76	.84	.071	.53	6.00	32.8	216.0	Green, few flowerheads.
January, 1934.....	.41	18.4	.44	3.82	.82	.074	.52	5.48	25.2	185.0	Green, with flowerheads.
February, 1934.....	.51	20.0	.39	2.97	.76	.040	.50	5.41	23.2	135.0	Green, short.
March, 1934.....	.38	15.1	.41	3.05	.86	.022	—	—	33.3	220.0	Green, with flowerheads.
<b>UROCHLOA PULULANS.</b>											
February, 1933.....	.50	17.7	.85	3.63	.97	.79	.98	—	18.3	45.0	Practically no growth.
March, 1933.....	.54	23.0	1.00	2.65	1.05	2.02	.61	8.22	28.6	50.0	Green, short.
April, 1933.....	.38	16.9	.83	2.61	.86	1.58	.65	6.22	25.5	83.0	Green, few flowerheads.
November, 1933.....	.38	10.7	.72	2.55	.60	.53	.54	5.23	31.4	100.0	Green, with flowerheads.
December, 1933.....	.46	11.0	.82	2.70	.61	.57	.57	6.12	29.9	77.0	Green, few flowerheads.
January, 1934.....	.44	11.8	.89	2.77	.70	.60	.57	6.07	28.0	26.0	Green, short.
February, 1934.....	.31	12.4	.77	2.97	.76	.028	.65	—	29.4	25.0	Green, short.
March, 1934.....	.37	15.3	.75	3.56	.86	.035	.72	—	28.0	45.0	Green, with flowerheads.
April, 1933.....	.32	13.7	.73	2.82	.79	.038	.70	—	26.6	45.0	Green, few flowerheads.
November, 1933.....	.30	12.6	.50	2.94	.65	.034	.39	4.89	37.9	53.0	Green, few flowerheads.
December, 1933.....	.34	13.4	.50	2.84	.65	.038	.37	4.80	37.7	53.0	Green, with flowerheads.
January, 1934.....	.30	11.0	.55	2.22	.60	.021	.37	3.85	36.1	55.0	Green, with flowerheads.
February, 1934.....	.42	13.6	.63	2.50	.76	.051	.43	4.64	35.4	56.0	Green, few flowerheads.
March, 1934.....	.35	12.8	.50	2.24	.73	.Trace	—	4.44	33.6	76.0	Green, with flowerheads.
<b>PANICUM MAXIMUM.</b>											
February, 1933.....	.38	18.1	.95	2.42	1.31	.96	.76	—	24.5	35.0	Practically no growth.
March, 1933.....	.36	23.1	1.20	2.18	1.23	1.24	—	6.70	23.8	50.0	Green, with flowerheads.
April, 1933.....	.28	8.2	.69	3.87	.70	1.09	.90	5.78	32.1	321.0	Green, short.
November, 1933.....	.25	9.2	.53	2.80	.57	.79	.87	5.04	30.9	160.0	Green, few flowerheads.
December, 1933.....	.34	12.0	.80	1.55	.80	.32	1.03	5.34	—	8.0	Green, with flowerheads.
January, 1934.....	.34	12.0	.80	1.55	.80	.32	1.03	5.34	—	8.0	Green, short.
February, 1934.....	.34	12.0	.80	1.55	.80	.32	1.03	5.34	—	8.0	Green, short.
March, 1934.....	.34	12.0	.80	1.55	.80	.32	1.03	5.34	—	8.0	Green, short.
<b>ERAGROSTIS SUPERA.</b>											
February, 1933.....	.31	11.3	.71	1.66	.60	.026	.37	—	28.0	90.0	Green, few flowerheads.
March, 1933.....	.36	13.3	.79	2.13	.60	.036	.44	—	25.0	135.0	Green, with flowerheads.
April, 1933.....	.32	12.6	.72	2.33	.60	.036	.44	—	25.0	135.0	Green, with flowerheads.
November, 1933.....	.32	12.6	.72	2.33	.60	.036	.44	—	25.0	135.0	Green, with flowerheads.
December, 1933.....	.28	10.2	.71	1.87	.53	.032	.20	4.43	30.9	120.0	Green, few flowerheads.
January, 1934.....	.27	7.1	.54	1.73	.38	.040	.11	3.94	37.8	37.8	Green, with flowerheads.
February, 1934.....	.29	12.5	.51	2.10	.38	.037	.08	3.27	38.5	190.0	Green, with flowerheads.
March, 1934.....	.37	13.0	.58	1.88	.47	.030	.19	4.56	31.9	140.0	Green, few flowerheads.
April, 1934.....	.37	13.0	.58	1.88	.47	.030	.19	4.56	31.2	104.0	Green, few flowerheads.

TABLE B.  
*Two-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>HYPARRHENIA HIRTA.</b>											
March, 1933.	.29	10.1	.55	2.09	45	.017	.59	—	31.7	270.0	Green, with flowerheads.
May, 1933.	.20	8.3	.76	1.86	53	.015	.69	3.66	45.4	110.0	Mixed, mainly green, with flowerheads.
November, 1933.	.35	15.2	.90	1.70	.45	.038	.46	3.78	33.2	130.0	Green, short.
January, 1934.	.14	5.1	.34	1.31	.30	.029	.17	2.12	48.9	910.0	Green, with flowerheads.
<b>THEMEDA TRIANDRA.</b>											
March, 1933.	.30	11.0	.75	1.59	44	.023	.56	—	28.4	95.0	Green, few flowerheads.
May, 1933.	.20	8.3	.83	1.12	.51	.009	.53	3.09	25.4	55.0	Mixed, mainly green, short.
November, 1933.	.29	11.3	.70	1.59	.39	.035	.36	3.29	35.6	100.0	Green, short.
January, 1934.	.15	6.2	.44	1.22	31	.044	.33	2.37	39.3	380.0	Green, with flowerheads.
<b>CYNBOPOGON PLURINOIDES.</b>											
March, 1933.	.36	11.0	.50	2.31	38	Trace	.55	—	25.0	80.0	Green, few flowerheads.
May, 1933.	.26	8.7	.58	2.29	46	.025	.59	4.03	25.0	25.0	Mixed, short.
November, 1933.	.35	16.5	.70	1.96	48	.041	—	3.68	27.0	15.0	Green, short.
January, 1934.	.19	7.5	.54	1.49	28	.047	.21	2.63	35.8	122.0	Green, with flowerheads.
<b>CYNODON DACTYLON.</b>											
March, 1933.	.24	12.9	.67	1.57	45	.024	.48	—	26.2	50.0	Green, with flowerheads.
May, 1933.	.15	9.0	.67	.60	.43	.017	.19	2.96	29.3	60.0	Mixed, mainly green, short.
November, 1933.	.31	14.4	.59	1.87	.36	.081	.32	3.91	29.1	120.0	Green, short.
January, 1934.	.18	9.4	.46	1.36	55	.084	.24	3.11	31.4	400.0	Green, with flowerheads.
<b>AMPHILOPHIS INSECTA.</b>											
March, 1933.	.24	9.3	.64	2.13	.46	.019	.83	—	32.0	390.0	Green, with flowerheads.
May, 1933.	.18	8.4	.80	1.47	.57	.037	.82	4.50	28.6	145.0	Mixed, mainly green, seeds falling out.
November, 1933.	.32	13.7	.86	1.93	.74	.036	.68	4.74	32.4	120.0	Green, short.
January, 1934.	.22	7.2	.39	1.77	.38	.041	.22	3.25	40.6	560.0	Green, with flowerheads.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE B. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>Pennisetum Cymare.</b>											
March, 1933.	.30	13.0	.42	3.62	.91	.084	1.19	—	31.3	410.0	Green, with flowerheads.
May, 1933.	.23	12.3	.85	1.80	1.04	.018	.97	4.87	25.9	100.0	Mixed, mainly green, short.
November, 1933.	.35	15.2	.43	3.85	.66	.11	.64	6.78	32.4	480.0	Green, few flowerheads.
January, 1934.	.30	10.3	.37	2.31	.81	.030	.56	4.39	35.2	890.0	Green, with flowerheads.
<b>Urochloa Puelulans.</b>											
March, 1933.	.49	15.9	.76	3.11	.83	.91	1.20	—	21.3	75.0	Green, short.
May, 1933.	.42	13.0	.74	2.44	.78	.65	.75	5.55	21.3	70.0	Mixed, short.
November, 1933.	.45	20.6	1.10	2.81	1.26	1.07	.60	7.86	23.6	76.0	Green, short.
January, 1934.	.23	7.3	.51	2.22	.55	.39	.79	4.78	35.2	430.0	Green, with flowerheads.
<b>Setaria Gerrardii.</b>											
March, 1933.	.33	13.7	.81	3.52	.89	Trace	1.02	—	27.7	60.0	Green, with flowerheads.
May, 1933.	.39	15.9	.72	3.08	.81	.045	.47	5.12	32.8	50.0	Practically no growth.
November, 1933.	.16	9.7	.31	1.82	.57	.028	.56	3.46	36.3	328.0	Green, short.
January, 1934.											Green, with flowerheads.
<b>Panicum Maximum.</b>											
March, 1933.	.37	17.2	.85	2.70	1.26	.89	1.00	—	23.3	140.0	Green, with flowerheads.
May, 1933.	.26	15.1	1.08	1.32	1.33	.82	.77	5.83	23.0	60.0	Mixed, mainly green, short.
November, 1933.	.30	23.9	1.11	2.49	1.12	1.39	.56	7.43	23.1	140.0	Green, short.
January, 1934.	.15	8.8	.51	1.43	.62	1.26	.64	4.34	36.5	460.0	Green, with flowerheads.
<b>Eragrostis Surereba.</b>											
March, 1933.	.29	11.4	.84	2.28	.74	.050	.71	—	26.8	172.0	Green, with flowerheads.
May, 1933.	.24	8.9	.85	1.40	.67	.013	.55	4.14	23.6	85.0	Mixed, mainly green, short.
November, 1933.	.27	12.1	.75	1.40	.69	.048	.22	4.29	33.3	246.0	Green, few flowerheads.
January, 1934.	.18	5.4	.46	1.37	.37	.036	.03	2.80	39.5	450.0	Green, with flowerheads.

TABLE C.  
*Three-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
April, 1933.....	·21	8·7	·67	1·88	·48	·015	61	—	28·0	250·0	Mixed, mainly green, few flowerheads.
January, 1934.....	·09	3·7	·26	1·00	·20	·013	14	1·64	50·8	1720·0	Green, with flowerheads.
April, 1933.....	·19	7·6	·70	1·17	40	NH	·40	—	29·6	255·0	Mixed, mainly green, few flowerheads.
January, 1934.....	·11	8·3	·46	89	34	·024	15	2·26	38·8	1270·0	Green, with flowerheads.
April, 1933.....	·29	9·9	·61	1·94	41	·018	·51	—	24·3	110·0	Mixed, mainly green, few flowerheads.
January, 1934.....	·15	5·8	·34	1·08	·33	·037	·19	2·00	40·0	510·0	Green, with flowerheads.
April, 1933.....	·18	11·1	·73	1·15	46	Trace	40	—	26·2	130·0	Green, short.
January, 1934.....	·13	7·0	·34	1·11	·36	·057	·19	2·62	35·5	650·0	Green, with seeds.
April, 1933.....	·21	9·1	70	1·68	·48	·019	62	—	27·7	250·0	Mixed, mainly green, few flowerheads.
January, 1934.....	·18	6·0	·39	1·56	·34	·035	21	2·83	40·6	920·0	Green, with flowerheads.
April, 1933.....	·21	9·7	·56	2·10	·83	·029	·85	—	31·0	395·0	Mixed, mainly green, seeds falling out.
January, 1934.....	·18	8·5	35	1·45	76	·025	·41	3·49	39·4	1360·0	Green, seeds falling out.
April, 1933.....	—	—	—	—	—	—	—	—	—	—	Mixed, seeds falling out.
January, 1934.....	·16	5·9	·54	1·84	47	·47	73	3·87	36·5	1780·0	Green, seeds falling out.
April, 1933.....	—	—	—	—	—	—	—	—	—	—	Mixed, mainly green, seeds falling out.
January, 1934.....	·15	7·0	·37	1·70	55	·025	·48	3·32	41·7	434·0	Green, with flowerheads.
April, 1933.....	—	—	—	—	—	—	—	—	—	—	Mixed, mainly green, seeds falling out.
January, 1934.....	11	5·4	·42	1·10	55	·80	·62	3·44	37·7	1240·0	Green, seeds falling out.
April, 1933.....	·21	8·7	67	1·50	·48	·035	·44	—	25·0	135·0	Mixed, mainly green, seeds falling out.
January, 1934.....	·15	4·3	·42	1·25	31	·016	·09	2·60	43·5	830·0	Green, seeds falling out.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE D.  
Four-monthly Cuttings.

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
May, 1933. January, 1934.	.20 .07	8.3 3.4	.73 .22	1.60 1.45	.49 .18	.017 .013	.54 .18	3.55 2.12	31.2 43.6	150.0 1430.0	Mixed, with flowerheads. Green, with flowerheads.
May, 1933. January, 1934.	.11 .065	5.2 4.8	.72 .46	.72 .75	.35 .36	.010 .022	.22 .24	2.41 1.71	29.1 40.8	220.0 1080.0	Mixed, few flowerheads. Green, with flowerheads.
May, 1933. January, 1934.	.26 16	11.4 5.6	.54 .38	1.63 1.19	.38 .24	.020 .017	.44 .26	3.51 2.11	27.1 41.4	130.0 500.0	Mixed, few flowerheads. Green, with flowerheads.
May, 1933. January, 1934.	.15 .13	8.4 6.5	.54 .39	.70 1.08	.39 .36	.017 .067	.33 .16	2.60 2.95	28.7 33.5	140.0 1000.0	Mixed, mainly green, few flowerheads. Green, with seeds.
May, 1933. January, 1934.	.19 .20	8.4 5.4	.75 .47	1.52 1.51	.52 .32	.016 .026	.67 .25	4.00 2.89	29.0 41.5	185.9 820.0	Mixed, seeds falling out. Green, with flowerheads.
May, 1933. January, 1934.	.16 .19	8.3 7.1	.66 .35	1.50 1.56	.79 .71	.018 .039	.64 .37	3.72 3.68	31.0 35.3	320.0 1210.0	Mixed, mainly green, seeds falling out. Green, seeds falling out.
May, 1933. January, 1934.	.42 .17	14.6 7.0	.98 .52	3.21 2.65	.84 .48	.26 .32	.79 .89	6.59 4.72	19.4 35.2	145.0 1100.0	Mixed, short, seeds falling out. Green, seeds falling out.
May, 1933. January, 1934.	.18 .17	8.9 7.8	1.18 .48	2.28 1.94	.94 .58	.016 .019	.61 .44	5.52 3.83	25.5 38.7	50.0 450.0	Mixed, mainly brown, seeds falling out. Green, with flowerheads.
May, 1933. January, 1934.	.18 .10	12.5 6.6	1.92 .49	1.91 1.24	1.23 .60	.75 .86	.64 .57	4.97 3.51	24.4 37.2	105.0 810.0	Mixed, seeds falling out. Green, seeds falling out.
May, 1933. January, 1934.	.20 .11	9.0 4.0	.79 35	1.36 1.08	.53 .26	.008 .019	.40 .08	4.14 2.26	28.5 46.3	150.0 800.0	Mixed, short, seeds falling out. Green, seeds falling out.

TABLE E.  
*Six-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	(Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
July, 1933. January, 1934.	.17 .15	8.4 8.4	.86 .85	.83 1.30	.60 .29	.015 .022	.37 .09	3.24 2.22	25.4 43.8	58.0 590.0	Brown, short. Green, with flowerheads.
July, 1933. January, 1934.	.10 .11	5.0 5.0	.74 .49	.70 .85	.45 .35	.012 .026	.27 .14	2.23 2.01	31.1 38.4	85.0 910.0	Brown, short. Green, with flowerheads.
July, 1933. January, 1934.	.18 .14	5.5 5.3	.40 .23	1.04 1.07	.27 .28	0.10 0.16	.27 .27	1.94 1.92	31.3 40.4	335.0 1030.0	Brown, seeds falling out. Green, with flowerheads.
July, 1933. January, 1934.	.12 .12	6.7 6.1	.70 .37	.56 1.15	.36 .28	.015 .056	.16 .13	3.05 2.73	29.2 35.6	70.0 1200.0	Brown, short. Green, with seeds.
July, 1933. January, 1934.	.18 .15	8.0 4.5	.82 .46	1.08 1.35	.50 .35	.015 .026	.57 .34	3.44 2.60	31.5 45.5	85.0 460.0	Brown, short. Green, with flowerheads.
July, 1933. January, 1934.	.14 .20	7.7 6.8	.80 .80	1.32 1.55	.70 .70	.025 .042	.47 .42	3.81 3.47	29.9 37.6	120.0 970.0	Brown, short, few seeds. Green, seeds falling out.
July, 1933. January, 1934.	.37 .19	11.9 8.1	.96 .44	1.96 2.96	.78 .51	.19 .42	.60 1.04	5.08 5.31	23.6 35.4	100.0 1280.0	Brown, short, few seeds. Green, seeds falling out.
July, 1933. January, 1934.	.18 .19	9.0 7.8	1.32 .53	1.32 1.36	.91 .55	.020 .020	.32 .33	4.33 2.97	27.6 41.8	40.0 130.0	Brown, short, few seeds. Green, with flowerheads.
July, 1933. January, 1934.	.18 .11	11.3 7.5	.95 .46	1.20 1.83	.98 .59	.54 .63	.71 .68	4.38 3.54	28.6 35.5	107.0 710.0	Brown, short, few seeds. Green, seeds falling out.
July, 1933. January, 1934.	.16 .11	7.3 4.7	.77 .36	1.09 1.15	.50 .29	.011 .022	.36 .09	3.33 2.41	29.6 44.0	110.0 800.0	Brown, short, seeds fallen out. Green, seeds falling out.



TABLE F.  
*Eight-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
May, 1933.....	10	4.1	36	1 10	27	.015	.27	2.13	33.6	895.0	Mixed, mainly brown, seeds falling out.
January, 1934.....	13	5.1	24	1.47	24	.024	.15	2 18	48 0	1680.0	Green, with flowerheads.
May, 1933.....	11	3.2	59	.78	.28	.009	.24	2 26	31.0	805 0	Mixed, mainly brown, seeds falling out.
January, 1934.....	12	3.9	48	.89	.26	.035	.09	1 75	41 0	2070 0	Green, with flowerheads.
May, 1933.....	11	4.3	39	.63	.23	.013	.18	2 04	34.3	870.0	Mixed, mainly brown, seeds falling out.
January, 1934.....	15	4.4	23	.99	26	.015	.16	1 74	46 9	1280.0	Green, with flowerheads.
May, 1933.....	13	6.2	59	.66	39	.018	.21	2.67	30 7	535 0	Mixed, mainly brown, seeds falling out.
January, 1934.....	14	6.1	34	1.17	.29	.049	.16	2 64	39.7	1420.0	Green, with seeds.
May, 1933.....	13	4.9	54	.89	36	.019	.36	3.12	33 2	730.0	Mixed, mainly brown, seeds fallen out.
January, 1934.....	17	6.3	48	1.12	.39	.027	.34	2 75	44.0	840.0	Green, seeds falling out.
May, 1933.....	19	5.5	43	1.50	.49	.017	.39	3 00	29.2	945.0	Mixed, seeds falling out.
January, 1934.....	26	6.2	27	1.70	.66	.031	.50	3 61	39 0	1750.0	Green, seeds falling out.
May, 1933.....	25	7.9	84	1.74	.60	.16	.57	4 24	27.5	845 0	Mixed, mainly brown, seeds falling out.
January, 1934.....	28	6.8	56	2.47	48	.26	.84	4.50	35 2	1350.0	Green, seeds falling out.
May, 1933.....	11	6.0	94	.87	.55	.012	.22	3 31	32.1	305.0	Brown, seeds falling out.
January, 1934.....	21	9.4	45	1.68	.53	.037	.36	3 28	40.9	104.0	Green, seeds falling out.
May, 1933.....	16	8.2	85	1 60	.95	.22	.52	4.73	28.1	845 0	Mixed, mainly brown, seeds falling out.
January, 1934.....	15	7.5	51	1 48	.90	.20	.74	3 91	38.1	1400.0	Green, seeds falling out.
May, 1933.....	107	4.4	55	.75	32	.010	.15	2 40	32.2	635.0	Mixed, mainly brown, seeds fallen out.
January, 1934.....	12	4.5	41	1 01	29	.023	.09	2.27	45.8	610.0	Green, seeds falling out.

TABLE (I).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	(aO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	(Cl.	Soluble ash.	(rude fibr.	Total yield in grams.	Description of cuts.
CHENOPODIUM MONTANA.											
March, 1933	50	15.3	57	2.10	50	0.50	80	—	25.7	85.0	Green, short.
April, 1933	46	14.7	54	2.03	51	0.46	80	—	28.7	76.0	Green, short.
May, 1933	45	16.0	52	2.03	54	0.51	80	3.55	27.9	26.0	Green, short.
August, 1933	37	13.4	58	1.66	55	0.81	36	3.37	27.0	25.0	Green, short.
September, 1933	40	14.4	62	1.80	56	0.86	44	3.46	27.4	23.0	Green, short.
October, 1933	29	13.7	67	1.41	49	0.70	42	3.81	31.5	65.0	Green, with flowerheads.
November, 1933	37	14.4	60	2.13	47	0.40	45	4.07	32.5	100.0	Green, short.
December, 1933	37	14.4	54	2.35	49	0.44	47	4.07	32.5	100.0	Green, short.
January, 1934	36	14.4	60	1.91	47	0.43	44	3.84	35.5	72.0	Green, short.
February, 1934	41	13.0	51	2.03	36	0.80	42	4.20	30.9	25.0	Green, short.
March, 1934	42	13.7	62	1.87	42	0.58	—	3.86	32.9	34.0	Green, few flowerheads.
ERAGROSTIS BRIZOIDES.											
March, 1933	31	12.7	54	1.88	39	0.28	60	—	30.2	145.0	Green, few flowerheads.
April, 1933	28	11.0	51	1.71	36	0.27	59	—	28.2	95.0	Green, few flowerheads.
May, 1933	—	10.9	46	1.20	31	0.41	—	2.75	28.2	20.0	Green, short.
November, 1933	25	11.3	50	1.64	38	0.41	33	2.95	38.6	120.0	Green, with flowerheads.
December, 1933	30	13.9	56	1.61	37	0.34	30	3.07	32.7	25.0	Green, short.
January, 1934	23	11.2	48	1.67	29	0.35	25	2.88	34.4	78.0	Green, with flowerheads.
February, 1934	31	12.5	57	1.55	37	0.30	25	3.14	31.8	85.0	Green, few flowerheads.
March, 1934	25	10.5	53	1.32	32	Trace	—	2.64	33.5	26.0	Green, short, few flowerheads.
ANTHEPHORA PUESCENS.											
March, 1933	39	15.4	75	2.20	1.13	0.21	1.15	—	31.8	135.0	Green, short.
April, 1933	37	11.0	65	1.82	0.99	0.19	1.05	—	34.5	195.0	Green, with flowerheads.
October, 1933	34	13.6	64	1.71	0.91	0.37	78	5.11	32.0	63.0	Green, few flowerheads.
November, 1933	36	12.8	55	2.61	1.31	0.55	76	5.60	34.4	240.0	Green, few flowerheads.
December, 1933	30	11.4	53	2.28	1.08	0.56	73	4.64	36.7	190.0	Green, with flowerheads.
January, 1934	28	11.5	57	2.46	0.90	0.61	69	4.81	25.5	340.0	Green, short.
February, 1934	39	11.8	61	2.12	0.86	0.45	66	5.88	32.9	75.0	Green, short, few flowerheads.
March, 1934	37	8.7	60	2.48	0.80	0.16	—	5.21	14.0	103.0	Green, short, few flowerheads.
ERAGROSTIS SP.											
March, 1933	43	14.2	51	2.53	37	Trace	1.01	—	31.6	150.0	Green, with flowerheads.
April, 1933	41	11.3	47	2.08	31	Trace	74	—	31.1	195.0	Green, with flowerheads.
August, 1933	41	16.0	70	1.72	49	0.86	36	3.40	25.4	82.0	Green, short.
September, 1933	39	14.6	68	1.62	52	0.87	57	3.65	28.7	100.0	Green, short.
October, 1933	33	13.4	66	1.34	37	0.84	41	3.40	29.4	73.0	Green, few flowerheads.
November, 1933	27	12.4	58	1.98	30	0.89	39	3.29	37.0	580.0	Green, with flowerheads.
December, 1933	32	14.4	57	1.74	39	0.80	43	3.26	32.8	124.0	Green, with flowerheads.
January, 1934	32	14.4	57	1.74	39	0.80	43	3.26	32.8	124.0	Green, with flowerheads.
February, 1934	26	11.2	45	1.74	32	0.84	44	3.53	34.0	164.0	Green, with flowerheads.
March, 1934	30	12.6	51	1.67	32	Trace	—	3.35	34.0	164.0	Green, with flowerheads.

## MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE G. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>PASPALUM DILATATUM.</b>											
March, 1933.	46	14.0	28	3.30	49	0.67	69	—	43.4	395.0	Green, with flowerheads.
April, 1933.	44	14.0	46	3.15	32	0.61	73	5.09	20.5	250.0	Green, short.
May, 1933.	44	14.5	51	2.70	22	0.59	75	4.72	23.0	85.0	Green, short.
August, 1933.	42	15.9	62	2.40	69	0.59	74	6.09	25.7	130.0	Green, short.
September, 1933.	48	16.7	60	3.30	51	0.62	63	5.46	28.3	130.0	Green, short.
October, 1933.	36	12.7	42	2.85	58	0.55	63	5.46	38.1	480.0	Green, with flowerheads.
November, 1933.	26	10.6	28	3.06	34	10	40	35.0	35.0	400.0	Green, with flowerheads.
December, 1933.	34	10.7	25	2.88	34	0.92	44	4.85	37.5	570.0	Green, with flowerheads.
January, 1934.	22	9.3	24	2.70	30	0.91	49	4.42	39.0	400.0	Green, with flowerheads.
February, 1934.	33	10.3	29	2.75	40	0.56	43	5.90	32.3	290.0	Green, with flowerheads.
March, 1934.	34	11.8	42	2.78	43	0.46	—	5.32	—	—	—
<b>ERAGROSTIS PLANA.</b>											
March, 1933.	39	12.5	47	1.88	22	0.27	72	—	36.1	195.0	Green, with flowerheads.
April, 1933.	34	12.3	48	1.32	21	0.52	52	—	33.7	105.0	Green, short.
October, 1933.	35	12.3	45	1.27	24	1.5	44	3.25	34.8	43.0	Green, short.
November, 1933.	32	11.0	47	1.70	24	1.0	41	3.12	36.9	515.0	Green, few flowerheads.
December, 1933.	17	8.6	36	1.87	19	0.88	37	3.13	38.5	180.0	Green, few flowerheads.
January, 1934.	26	10.5	35	1.61	19	0.82	37	2.91	40.5	440.0	Green, with flowerheads.
February, 1934.	26	10.5	36	1.66	14	0.53	49	3.26	39.4	135.0	Green, with flowerheads.
March, 1934.	30	11.4	39	1.36	18	0.28	—	2.53	41.3	40.0	Green, short, few flowerheads.
<b>CHLORIS GAYANA.</b>											
March, 1933.	47	15.7	66	2.96	40	1.20	162	—	29.8	190.0	Green, with flowerheads.
April, 1933.	40	14.5	77	2.22	37	1.26	172	—	28.4	210.0	Green, few flowerheads.
May, 1933.	41	16.1	82	1.67	44	1.20	162	6.23	24.8	25.0	Green, short.
November, 1933.	33	16.5	68	2.65	36	1.62	86	6.90	30.2	130.0	Green, short.
December, 1933.	34	16.1	76	2.10	45	1.25	86	5.94	26.8	80.0	Green, short.
January, 1934.	20	10.4	46	1.49	25	0.98	108	4.76	41.3	190.0	Green, few flowerheads.
February, 1934.	27	11.8	70	1.54	19	0.95	109	5.27	26.1	87.0	Green, few flowerheads.
March, 1934.	38	12.5	80	1.69	32	0.89	—	5.39	29.6	100.0	Green, with flowerheads.
<b>ALLOTROPIS SEMIALATA.</b>											
March, 1933.	63	14.5	25	2.50	33	Trace	62	—	38.4	85.0	Green, with flowerheads.
April, 1933.	58	15.7	32	2.46	36	Trace	57	—	37.7	50.0	Green, with flowerheads.
September, 1933.	68	15.8	31	2.68	42	Trace	63	4.58	—	10.0	Green, short.
October, 1933.	37	12.1	30	2.37	42	0.81	46	4.37	38.4	15.0	Green, few flowerheads.
November, 1933.	39	16.1	27	2.48	37	0.60	45	3.33	37.6	35.0	Green, short.
December, 1933.	41	16.2	27	2.48	37	0.60	45	3.32	37.7	38.0	Green, short.
January, 1934.	44	17.2	29	2.48	37	0.63	44	3.32	40.3	62.0	Green, short.
February, 1934.	44	17.0	25	2.73	36	0.62	43	4.24	40.2	55.0	Green, short, few flowerheads.
March, 1934.	50	15.3	29	2.75	37	0.72	—	3.96	40.8	—	—

TABLE 6. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein	CaO.	K <sub>2</sub> O	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<b>ELANURUS ARGENTUS</b>											
March, 1933.....	46	14.3	45	2.48	36	034	50		29.7	190.0	Green, short.
April, 1933.....	41	13.0	51	2.48	39	034	40		26.7	135.0	Green, short.
May, 1933.....	44	13.4	53	2.21	43	038	39	1.96	25.7	45.0	Green, short.
August, 1933.....	42	13.5	73	1.64	51	037	45	1.13	21.7	22.0	Green, short.
September, 1933.....	40	12.6	78	1.93	47	031	47	3.72	96	18.0	Green, with flowerheads.
October, 1933.....	27	11.4	68	1.47	49	039	46	3.67	51.6	150.0	Green, few flowerheads.
November, 1933.....	32	13.9	51	2.10	38	039	49	3.46	30.9	170.0	Green, with flowerheads.
December, 1933.....	30	12.2	48	2.15	37	040	45	3.16	32.4	170.0	Green, short.
January, 1934.....	32	16.9	53	1.78	34	035	42	3.46	29.9	160.0	Green, few flowerheads.
February, 1934.....	35	12.0	50	2.22	44	028	40	3.50	28.4	118.0	Green, short
March, 1934.....	33	13.2	47	1.92	48	029	40				
<b>ISOBREMIA GLAUCOSTACHYUM</b>											
March, 1933.....	28	9.5	59	1.31	42	016	46		32.6	215.0	Green, with flowerheads.
April, 1933.....	27	10.0	68	1.29	47	014	52		30.5	100.0	Green, short.
May, 1933.....											Practically no growth.
November, 1933.....	21	8.8	56	1.83	35	041	35	3.06	35.0	127.0	Green, with flowerheads.
December, 1933.....	25	10.3	57	1.51	35	038	32	2.87	37.0	140.0	Green, few flowerheads.
January, 1934.....	17	8.0	47	1.51	35	047	21	2.93	37.3	140.0	Green, with flowerheads.
February, 1934.....	20	7.4	47	1.11	38	012	19	2.54	35.6	315.0	Green, with flowerheads.
March, 1934.....	26	9.4	55	1.36	38	011		3.18	34.7	109.0	Green, short, few flowerheads.
<b>POGONANTHERA FALCATA</b>											
March, 1933.....	45	15.9	58	1.29	49	034	39		30.9	45.0	Green, with flowerheads.
April, 1933.....	34	13.6	54	1.35	41	023	37		29.8	55.0	Green, few flowerheads.
May, 1933.....	31	12.1	52	1.66	37	020	32	2.61		25.0	Green, short.
November, 1933.....	29	14.7	61	1.79	38	075	24	3.46	32.3	46.0	Green, short.
December, 1933.....	30	13.9	54	1.56	35	050		2.70	34.3	23.0	Green, short.
January, 1934.....	11.9	39	39	1.67	29	072	19	2.80	35.7	100.0	Green, few flowerheads.
February, 1934.....	25		45		32	026		2.93	35.0	29.0	Green, few flowerheads.
March, 1934.....	31	12.5		1.38							Practically no growth.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE H.  
*Two-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<i>ISCHAEMUM GLAUCOSTACHYUM.</i>											
April, 1933.....	16	6.4	65	92	42	012	38	—	34.4	195.0	Mixed, mainly green, few flowerheads.
December, 1933. ....	17	5.0	50	130	37	032	34	2.57	42.1	263.0	Green, with flowerheads.
February, 1934.....	17	6.0	39	85	33	014	46	2.14	36.9	252.0	Green, with flowerheads.
<i>POGONARTHRIA FALCATA.</i>											
April, 1933.....	27	10.2	55	125	38	025	45	—	31.7	70.0	Green, with flowerheads.
December, 1933.....	27	11.0	38	167	33	054	24	2.88	36.1	308.0	Green, with flowerheads.
February, 1934.....	19	6.9	29	109	22	029	15	2.09	46.5	490.0	Green, with flowerheads.
<i>CHRYSOPOGON MONTANA.</i>											
April, 1933.....	36	12.9	69	194	50	029	69	—	27.9	175.0	Green, few flowerheads.
June, 1933.....	33	13.1	68	210	74	034	48	4.41	27.5	45.0	Mixed, short.
August, 1933.....	36	12.9	56	180	54	035	37	3.65	27.6	35.0	Green, short.
October, 1933.....	23	11.4	86	128	66	021	29	3.63	25.6	80.0	Green, short.
December, 1933.....	28	9.4	48	160	57	033	44	3.25	33.7	670.0	Green, with flowerheads.
February, 1934.....	24	9.7	34	152	29	018	50	3.37	32.1	250.0	Green, with flowerheads.
<i>ERAGROSTIS BRIZOIDES.</i>											
April, 1933.....	19	8.8	33	139	35	021	52	—	27.7	210.0	Green, with flowerheads.
December, 1933.....	18	6.3	33	123	24	036	22	2.13	30.2	408.0	Green, with flowerheads.
February, 1934.....	16	8.0	31	120	24	023	20	2.08	40.0	605.0	Green, with flowerheads.
<i>ANTHROPORA PUBESCENS.</i>											
April, 1933.....	21	9.3	87	142	129	014	104	—	33.4	400.0	Green, with flowerheads.
October, 1933.....	25	11.8	46	146	96	031	73	5.03	27.7	100.0	Green, few flowerheads.
December, 1933.....	20	7.3	46	150	103	082	69	3.69	38.5	930.0	Green, seeds falling out.
February, 1934.....	24	10.0	51	168	109	025	61	4.23	42.6	890.0	Green, with flowerheads.
<i>ERAGROSTIS SP.</i>											
April, 1933.....	28	10.0	52	178	30	Trace	67	—	30.0	260.0	Mixed, mainly green, seeds falling out.
August, 1933.....	38	6.2	64	163	36	035	34	3.13	26.1	30.0	Green, short.
October, 1933.....	25	10.8	66	147	31	019	58	3.42	28.9	50.0	Green, few flowerheads.
December, 1933.....	16	8.1	32	159	27	032	38	2.98	44.8	1040.0	Green, with flowerheads.
February, 1934.....	20	6.7	39	132	22	018	32	2.56	41.3	1100.0	Green, with flowerheads.

TABLE II. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	(rude fibre.	Total yield in grams.	Description of cuts.
<b>PASPALUM DILATATUM.</b>											
April, 1933.....	29	11.1	45	2.75	.51	.651	64	—	28.5	520 0	Green, seeds falling out.
June, 1933.....	32	10.4	68	2.42	.65	.651	80	5.72	23.8	85 0	Green, short.
October, 1933.....	27	10.5	56	2.32	.55	.649	45	4.74	23 6	165 0	Green, short.
December, 1933.....	22	8.1	26	2.55	.16	.681	40	3.96	43 8	760 0	Green, with flowerheads.
February, 1934.....	14	6.8	21	2.55	.37	.641	60	4.38	39.1	1000 0	Green, with flowerheads.
<b>ERAGROSTIS PLANA.</b>											
April, 1933.....	24	9.6	50	1.50	.19	.035	63	—	29.8	155 0	Mixed, mainly green. seeds falling out.
December, 1933.....	19	8.6	38	1.82	.19	.081	35	3.17	39.7	1000 0	Green, with flowerheads.
February, 1934.....	19	6.0	34	1.48	.13	.046	29	2.63	43 0	850 0	Green, with flowerheads.
<b>CHLORIS GAYANA.</b>											
April, 1933.....	29	10.0	73	1.44	.37	1.32	1.71	—	33 0	685 0	Green, with flowerheads.
December, 1933.....	24	16.0	61	1.96	.32	1.17	1.08	5.65	33.4	740 0	Green, with flowerheads.
February, 1934.....	16	8.3	40	1.22	.24	1.03	.99	4.45	37.5	830 0	Green, with flowerheads.
<b>ALLOTROPIS SEMIALATA.</b>											
April, 1933.....	56	13.6	42	2.27	.42	Trace	.60	—	36.8	50 0	Green, with flowerheads.
October, 1933.....	48	13.9	45	2.59	.22	.088	.72	4.69	36.1	18 0	Green, short.
December, 1933.....	27	10.2	28	2.52	.29	.073	.45	3.59	40.8	220 0	Green, with flowerheads.
February, 1934.....	37	10.8	55	2.48	.21	.039	.41	3.70	43.6	240 0	Green, with flowerheads.
<b>ELYNATUS ARGENTUS.</b>											
April, 1933.....	31	10.9	67	1.86	.43	.027	.56	—	27.4	260 0	Green, with flowerheads.
June, 1933.....	29	10.2	78	1.81	.54	.018	.50	3.84	22.4	55 0	Green, short.
August, 1933.....	43	13.0	70	2.06	.19	.037	.39	3.82	20.7	22 0	Green, short.
October, 1933.....	21	8.9	56	1.81	.41	.023	.43	4.06	24.2	75 0	Green, few flowerheads.
December, 1933.....	21	7.4	40	1.80	.30	.041	.32	3.03	33.5	650 0	Green, with seeds.
February, 1934.....	16	8.8	19	1.54	.33	.036	.30	3.81	31.5	500 0	—

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE I.  
*Three-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
ISCHAEMUM GLAUCOSTACHYUM.											
May, 1933.....	.11	5.4	.83	.66	.41	.013	.34	2.84	33.2	170.0	Mixed, with seeds.
November, 1933...	.17	9.5	.64	1.66	.36	.049	.37	2.96	36.8	123.0	Green, with flowerhead-
February, 1934...	.08	8.5	.37	.82	.23	.019	.16	2.01	38.8	390.0	Green, with seeds.
POGONATHERIA FALCATA.											
May, 1933.....	.20	9.3	.56	.85	.31	.011	.29	2.21	31.0	105.0	Mixed, mainly green, seed-
November, 1933....	.21	14.7	.67	1.69	.38	.052	.25	3.43	41.0	121.0	falling out.
February, 1934.....	.11	5.7	.20	1.00	.21	.017	.17	1.74	39.2	520.0	Green, short.
CHRYSOPOGON MONTANA.											
May, 1933.....	.25	11.6	.87	1.15	.85	.020	.19	4.55	27.0	145.0	Green, few seeds.
August, 1933.....	.36	12.5	.59	2.00	.62	.040	.56	4.80	27.0	30.0	Green, short.
November, 1933....	.28	11.8	.72	2.08	.52	.040	.51	4.44	34.2	230.0	Green, with flowerhead.
February, 1934...	.21	7.7	.50	1.12	.49	.018	.38	2.76	34.3	520.0	Green, with flowerhead.
ERAGROSTIS BRIZOIDES.											
May, 1933.....	.13	7.2	.52	.95	.36	.020	—	2.43	26.4	100.0	Mixed, few stalks, seeds falling
November, 1933....	.27	8.8	.43	1.63	.29	.047	.29	2.87	25.7	114.0	out.
February, 1934....	.14	7.1	.34	1.12	.22	.032	.20	2.22	36.1	360.0	Green, with flowerheads.
ANTHEPHORA PUBESCENS.											
May, 1933.....	.12	6.8	1.02	1.11	1.39	.024	.77	4.05	29.0	150.0	Mixed, seeds falling out.
November, 1933....	.31	11.4	.57	2.15	1.24	.048	.60	4.93	37.0	330.0	—
February, 1934...	.20	7.2	.45	1.36	.92	.040	.50	3.60	38.1	460.0	Green, with flowerheads.
ERAGROSTIS SP.											
May, 1933.....	.20	8.1	.49	1.44	.29	.022	.55	2.81	30.0	250.0	Mixed, seeds falling out
November, 1933....	.28	10.4	.30	2.07	.22	.043	.26	3.15	38.6	600.0	Green, with flowerheads.
February, 1934....	.16	5.7	.26	1.16	.18	.018	.23	2.18	39.2	560.0	Mixed, mainly green, seeds
											falling out.

TABLE I (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub>	Crude Protein	CaO	K <sub>2</sub> O	MgO	Na <sub>2</sub> O	Cl	Soluble ash	Crude fibre	Total yield in grams.	Description of cuts.
<b>PASPALUM DILATATUM</b>											
May, 1933.....	19	5.9	46	2.31	45	0.57	52	4.24	30.3	525.0	Mixed, mainly green, seeds falling out.
November, 1933...	33	16.6	50	3.47	54	0.80	44	5.90	37.0	250.0	Green, few flowerheads.
February, 1934...	14	6.6	27	2.28	41	0.47	53	4.31	40.9	780.0	Mixed, mainly green, seeds falling out.
<b>ERAGROSTIS PLANA.</b>											
May, 1933.....	17	6.9	55	1.14	21	0.16	61	2.65	33.8	220.0	Mixed, seeds falling out.
November, 1933...	19	10.0	44	1.77	20	0.80	32	2.93	38.9	490.0	Green, few flowerheads.
February, 1934...	10	5.0	26	1.22	12	0.45	30	2.13	41.3	700.0	Mixed, mainly green, seeds falling out.
<b>CHLOERIS GAYANA.</b>											
May, 1933 . . . . .	18	7.5	68	0.90	36	1.10	155	4.65	30.3	560.0	Mixed, mainly green, seeds falling out.
November, 1933..	27	13.9	66	2.15	38	1.41	117	6.00	29.6	250.0	Green, few flowerheads.
February, 1934..	12	6.2	41	1.16	29	69	88	3.75	36.0	740.0	Green, seeds falling out.
<b>ALLOTROPIS SEMIALATA.</b>											
May, 1933. . . . .	37	10.9	45	2.05	52	0.42	74	3.76	36.6	50.0	Green, few flowerheads.
November, 1933..	35	12.6	42	2.38	46	0.60	45	3.84	40.8	224.0	Green, few flowerheads.
February, 1934..	22	9.1	48	2.28	29	0.27	45	3.10	19.9	520.0	Green, with flowerheads.
<b>ELYONURUS ARGENTULUS.</b>											
May, 1933.....	20	7.1	79	1.29	49	0.23	50	3.51	27.2	235.0	Mixed, mainly green, few flowerheads.
August, 1933.....	32	12.1	60	3.17	38	NH	45	3.79	—	30.0	Green, short.
November, 1933..	25	9.4	38	2.48	26	0.76	39	3.62	38.0	450.0	Green, with flowerheads.
February, 1934....	16	7.0	41	1.49	35	0.06	22	2.87	33.5	170.0	Green, with flowerheads.



MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE J.  
*Four-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<i>ISCHAEMUM GLAUCOSTACHYUM</i>											
June, 1933.....	.10	4.6	.90	.58	.41	.011	.24	2.75	31.0	130.0	Mixed, mainly brown, seeds falling out.
February, 1934...	.08	4.5	.37	.57	.28	.010	.14	1.75	40.7	717.0	Mixed, mainly green, seeds falling out.
<i>POGONATHERA FALCATA.</i>											
June, 1933.....	.16	7.1	.55	.53	.25	.011	.23	1.97	31.5	115.0	Mixed, mainly brown, seeds falling out.
February, 1934.....	.15	6.0	.25	.99	.21	.017	.18	1.85	42.4	1770.0	Green, with flowerheads
<i>CHRYSOPOGON MONTANA.</i>											
June, 1933.....	.16	7.8	.97	.89	.94	.021	.36	3.34	26.6	135.0	Mixed, mainly brown, seeds falling out.
October, 1933.....	.13	10.0	.95	1.10	.82	.020	.27	3.79	36.4	70.0	Green, short.
February, 1934.....	.17	5.8	.54	.93	.47	.021	.31	2.63	36.5	1500.0	Green, seeds falling out.
<i>ERAGROSTIS BRIZOIDES.</i>											
June, 1933.....	.09	6.2	.58	.78	.37	.018	.38	2.43	29.2	75.0	Mixed, mainly brown, seeds falling out.
February, 1934.....	.12	6.0	.28	.98	.24	.016	.18	1.81	39.2	845.0	Green, with flowerheads
<i>ANTHROPORA PUBESCENS.</i>											
June, 1933.....	.10	6.0	1.01	.73	1.22	.024	.54	3.77	31.1	120.0	Mixed, mainly brown, seeds falling out.
October, 1933.....	.27	12.0	.70	2.23	.79	.029	.89	5.06	29.6	40.0	Green, short, few flowerheads.
February, 1934.....	.16	5.7	.42	1.37	.82	.015	.47	3.71	41.1	1,050.0	Mixed, mainly green, seeds falling out.

TABLE J (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	CaO.	K <sub>2</sub> O.	MgO	Na <sub>2</sub> O	Cl	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
ERAGROSTIS SP.										
June, 1933.....	16	47	1 24	27	023	41	2 68	33.8	205 0	Mixed, mainly brown, seeds falling out.
February, 1934.....	13	22	1 00	16	017	15	2 08	41.4	1440 0	Mixed, mainly green, seeds falling out.
PASPALUM DILATATUM.										
June, 1933.....	19	56	2 15	55	056	48	4 10	28.4	510 0	Mixed, seeds falling out.
February, 1934.....	11	26	2 15	41	019	45	4 07	39 0	1380 0	Mixed, mainly green, seeds falling out.
ERAGROSTIS PLANA.										
June, 1933.....	14	53	1 15	19	025	62	2 68	34.2	225 0	Mixed, seeds falling out.
February, 1934.....	09	29	1 26	12	054	18	2 21	40.8	2260 0	Mixed, mainly green, seeds falling out.
CHLOERIS GAYANA.										
June, 1933.....	13	74	49	34	1 10	122	3 98	31.4	370 0	Mixed, mainly brown, seeds falling out.
February, 1934.....	16	54	—	33	55	61	3 89	36.5	940 0	Green, seeds falling out.
ALLOTROPIS SEMIARATA.										
June, 1933.....	19	67	68	68	024	55	2 74	39.5	55 0	Mixed, mainly brown, seeds falling out.
October, 1933.....	30	42	2 47	35	030	1 12	4 48	36.3	50 0	Green.
February, 1934.....	16	23	1 82	27	014	57	3 01	43.7	900 0	Green, seeds falling out.
ELYONURUS ARGENTUS.										
June, 1933.....	12	84	80	51	008	37	3 03	28.7	260 0	Mixed, seeds falling out.
October, 1933.....	20	85	1 69	33	027	44	3 75	23.1	70 0	Green, few flowerheads.
February, 1934.....	11	33	95	21	018	12	1 50	39.2	1300 0	—

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE K.  
Five-monthly Cuttings.

Date of cutting.	P.O.	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
July, 1933.....	-10	4.5	85	.57	.40	.012	.25	2.85	30.9	160.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-16	6.5	.49	1.14	.29	.038	.32	2.61	39.6	315.0	Green, with flowerheads.
July, 1933.....	-15	7.4	.70	.60	.35	.015	.28	2.07	26.2	130.0	Mixed, seeds falling out.
December, 1933....	-19	3.4	—	1.33	.27	.065	.28	2.90	38.0	710.0	Green, with flowerheads.
July, 1933.....	-15	6.7	.94	1.00	.85	.018	.37	3.03	26.6	175.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-21	6.9	.49	1.83	.46	.034	.42	3.19	36.9	845.0	Green, with flowerheads.
July, 1933.....	-07	5.3	.62	.72	.39	.017	.27	2.54	29.5	115.0	Brown, seeds fallen out.
December, 1933....	-14	6.9	.36	1.19	.27	.034	.19	2.19	37.4	400.0	Green, with flowerheads.
July, 1933.....	-086	4.8	1.09	.65	1.18	.023	.50	3.93	31.9	185.0	Brown, seeds fallen out.
December, 1933....	-14	6.8	.40	1.64	1.02	.039	.53	3.46	42.2	760.0	Green, seeds falling out.
July, 1933.....	-16	6.7	.45	1.12	.29	.025	.39	2.28	32.8	285.0	Brown, seeds falling out.
December, 1933....	-13	6.8	.34	1.45	.23	.038	.27	2.57	41.5	710.0	Green, with flowerheads.
July, 1933.....	-11	4.4	.49	1.95	.47	.039	.44	4.08	32.2	560.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-24	8.7	.25	2.54	.35	.062	.43	4.15	42.3	930.0	Green, with flowerheads.
July, 1933.....	076	5.6	.61	.99	.20	.044	.56	2.72	37.4	162.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-14	5.7	.36	1.53	.39	.081	.34	2.68	39.1	660.0	Green, with flowerheads.
July, 1933.....	-08	4.9	.69	.62	.38	1.25	1.30	4.43	33.3	500.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-17	10.3	.43	1.91	.26	1.00	1.16	5.28	40.0	650.0	Green, with flowerheads.
July, 1933.....	-15	5.2	.72	.72	.66	.026	.46	3.03	37.5	65.0	Brown, seeds falling out.
December, 1933....	-29	8.8	.23	2.63	.27	.049	.40	3.57	43.4	560.0	Green, with flowerheads.
July, 1933.....	11	4.4	1.07	.67	.67	.021	.35	3.29	27.8	240.0	Mixed, mainly brown, seeds falling out.
December, 1933....	-16	6.3	.37	1.41	.26	.065	.31	2.71	26.9	940.0	Green, with seeds.

TABLE I.  
*Six-monthly Cuttings.*

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO	K <sub>2</sub> O	MgO.	Na <sub>2</sub> O	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cut.
<i>ISCHAEMUM CHAETOSTACHYUM.</i>											
August, 1933....	0.95	3.5	9.1	4.8	1.8	0.13	26	2.50	37.8	120.0	Mixed, mainly brown seeds.
February, 1934..	1.0	4.6	4.6	5.9	2.6	0.23	16	1.68	43.5	840.0	Mixed, mainly green, seeds falling out.
<i>POGONARTHRIA FALCATA</i>											
August, 1933....	1.12	6.3	6.4	6.8	7.0	0.17	3	2.11	32.2	80.0	Mixed, mainly brown, seeds falling out.
February, 1934.....	1.11	5.2	2.3	9.1	2.2	0.15	18	1.90	41.7	1185.0	Green, with flowerheads.
<i>CHRYSOPOGON MONTANA.</i>											
August, 1933....	1.14	8.6	8.4	5.2	4.9	0.13	11	2.23	27.1	110.0	Mixed, seeds falling out.
February, 1934..	1.13	5.8	4.3	1.01	4.5	0.16	28	2.47	35.7	1400.0	Green, seeds falling out.
<i>ERAGROSTIS BRIZOIDES.</i>											
August, 1933....	0.69	5.2	6.5	6.3	3.8	0.21	32	2.22	28.8	70.0	Brown, seeds, fallen out.
February, 1934..	1.2	5.9	3.0	6.6	2.5	0.20	18	2.04	38.9	725.0	Green, with flowerheads.
<i>ANTHROPORA PILESIFENS.</i>											
August, 1933....	0.8	4.4	1.01	6.8	1.32	0.24	48	3.54	34.8	145.0	Mixed, mainly brown, seeds falling out.
February, 1934..	1.3	5.1	4.6	1.20	8.1	0.27	15	3.48	38.0	1060.0	Mixed, mainly green, seeds falling out.
<i>ERAGROSTIS SP.</i>											
August, 1933....	1.15	6.4	5.8	9.8	2.9	0.17	45	2.50	33.1	192.0	Mixed, mainly brown, seeds falling out.
February, 1934..	1.10	4.5	2.4	9.5	2.9	0.15	15	2.23	40.8	1440.0	Mixed, mainly green, seeds falling out.
<i>PASPALUM FLUATUUM.</i>											
August, 1933....	1.2	4.3	6.3	18.3	5.2	0.16	43	4.00	33.7	400.0	Mixed, mainly brown, seeds falling out.
February, 1934..	1.13	5.2	2.1	2.01	3.6	0.38	39	3.91	39.4	920.0	Mixed, mainly green, seeds falling out.
<i>ERAGROSTIS PLANA</i>											
August, 1933....	0.87	5.4	5.2	1.68	1.8	0.18	58	2.75	37.8	145.0	Mixed, mainly brown, seeds falling out.
February, 1934..	0.8	3.9	2.7	1.23	10	0.21	33	2.39	41.9	1730.0	Mixed, mainly green, seeds falling out.
<i>CHLORIS GAYANA.</i>											
August, 1933....	1.2	5.3	8.1	6.0	3.8	1.13	149	4.61	30.5	450.0	Mixed, mainly brown, seeds falling out.
February, 1934..	1.6	7.1	5.9	1.29	3.9	0.45	72	3.93	35.9	950.0	Green, seeds falling out.
<i>ALLOTROPIS SEMIALATA.</i>											
August, 1933....	2.4	5.6	7.1	1.04	6.5	0.33	59	2.96	36.2	100.0	Mixed, mainly brown, seeds falling out.
February, 1934.....	1.7	6.6	2.1	1.73	1.1	0.17	32	2.74	46.6	1140.0	Green, seeds falling out.
<i>ELYNRIUS ARGENTUS.</i>											
August, 1933....	1.3	5.3	1.03	7.8	6.2	0.21	31	3.17	28.0	152.0	Mixed, mainly brown, seeds falling out.
February, 1934.....	1.10	5.0	3.6	9.6	2.3	0.22	16	2.30	34.9	1000.0	Green, seeds falling out.

TABLE M.  
7-, 8-, 9-, 10-, 11-, and 12-monthly Cuttings.

Date of cutting.	P <sub>2</sub> O <sub>5</sub> .	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	Na <sub>2</sub> O.	Cl.	Soluble ash.	Crude fibre.	Total yield in grams.	Description of cuts.
<i>ISCHAEUM GLAUCOSTACHYUM.</i>											
September, 1933.....	.10	4.8	.68	.46	.28	.007	.11	2.25	33.6	120 0	Mixed, mainly brown, seeds fallen out.
October, 1933.....	.09	4.4	.68	.40	.22	.017	.08	2.14	35.5	100.0	Brown, seeds fallen out.
November, 1933.....	.12	7.3	.57	1.08	.28	.029	.13	2.40	38.4	250.0	Mixed, mainly green, with flowerheads.
December, 1933.....	.12	5.7	.59	.81	.30	.032	.17	2.29	40.0	250 0	Mixed, mainly green, with flowerheads.
January, 1934.....	.10	4.2	.38	.72	.27	.017	.09	1.75	38.5	700.0	Mixed, mainly, green seeds falling out.
February, 1934.....	.10	3.7	.45	.48	.31	.008	.06	1.80	40 6	2600 0	Mixed, mainly green, seeds falling out.
<i>POGONATHERA FALCATA.</i>											
September, 1933.....	.15	7.6	.56	.46	.29	.008	.16	1.80	33.6	148 0	Mixed, mainly brown, seeds fallen out.
October, 1933.....	.11	7.2	.38	.59	.24	.020	.20	2.05	30.4	133.0	Mixed, seeds fallen out.
November, 1933.....	.16	9.8	.57	1.53	.32	.038	.26	2.75	33.8	640 0	Mixed, mainly green, seeds falling out.
December, 1933.....	.20	7.7	.36	1.44	.25	.034	.32	2.58	40.4	800 0	Mixed, mainly green, with flowerheads.
January, 1934.....	.16	6.2	.23	1.20	.20	.030	.18	2.13	40.5	1800.0	Green, with seeds.
February, 1934.....	.17	5.9	.24	.82	.20	.016	.11	1.95	40 9	2900 0	Mixed, mainly green, seeds falling out.
<i>CHRYSOPOGON MONTANA.</i>											
September, 1933.....	.15	8.6	.55	.92	.44	.014	.26	2.74	30.4	240 0	Mixed (stalks blown away).
October, 1933.....	.13	8.6	.87	.75	.58	.017	.22	3.07	26 0	180.0	Mixed, mainly green, few flowerheads.
November, 1933.....	.21	8.6	.62	1.55	.38	.034	.41	3.40	32.5	650.0	Green, with flowerheads.
December, 1933.....	.21	6.8	.50	1.77	.47	.040	.40	3.13	34.8	720.0	Green, seeds falling out.
January, 1934.....	.16	6.0	.39	1.16	.40	.015	.27	2.66	33.9	1700.0	Mixed, mainly green, seeds falling out.
February, 1934.....	.17	5.7	.37	1.00	.38	.043	.32	2.40	31 0	2000.0	Mixed, mainly green, seeds falling out.
<i>ERAGROSTIS BRIZOIDES.</i>											
September, 1933.....	.09	5.6	.45	.92	.21	.018	.08	1.44	31.6	73 0	Brown (stalks blown away).
October, 1933.....	.06	5.1	.48	.22	.17	.027	.05	1.81	35.0	73 0	Mixed, mainly green, few flowerheads.
November, 1933.....	.26	10.8	.43	1.42	.25	.037	.24	3.00	36.3	146 0	Mixed, mainly green, with flowerheads.
December, 1933.....	.19	7.2	.37	1.23	.32	.035	.22	2.18	39.8	250 0	Mixed, mainly green, with flowerheads.
January, 1934.....	.14	6.2	.39	1.15	.20	.026	.16	2.08	36.6	520.0	Green, seeds falling out.
February, 1934.....	.14	6.7	.36	.97	.21	.020	.14	2.15	35.2	840.0	Green, with flowerheads.

TABLE M. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub>	Crude Protein	CaO.	K <sub>2</sub> O	MgO	Na <sub>2</sub> O.	Cl	Soluble ash.	Crude fibre	Total yield in grams.	Description of cuts.
ANTHELIOPORA PUBESCENS											
September, 1933.	12	6.4	1.22	7.8	7.9	0.11	2.5	3.29	32.0	140.0	Mixed, mainly brown, seeds fallen out.
October, 1933.	09	6.1	9.5	5.3	5.0	0.21	1.8	3.19	30.0	170.0	Mixed, mainly brown, seeds fallen out.
November, 1933	22	8.8	5.4	1.85	9.7	0.37	5.6	4.41	36.7	620.0	Mixed, mainly green, seeds falling out.
December, 1933.	15	6.3	4.4	1.51	9.4	0.45	5.0	3.46	38.3	860.0	Green, seeds falling out.
January, 1934.	13	7.2	3.5	1.48	7.2	0.59	4.6	3.33	38.2	1360.0	Mixed, mainly green, seeds falling out.
February, 1934.	14	7.4	4.3	1.59	9.3	0.80	1.7	3.68	39.4	6000.0	Mixed, mainly green, seeds falling out.
ERAGROSTIS SP.											
September, 1933.	15	6.4	4.4	7.1	2.5	0.12	1.0	1.82	31.0	185.0	Mixed, mainly brown, seeds falling out.
October, 1933.	11	5.3	4.3	5.1	2.3	0.15	1.8	2.43	30.3	190.0	Mixed, mainly brown, seeds fallen out.
November, 1933.	25	9.9	3.0	1.88	2.3	0.48	3.4	2.94	40.9	1000.0	Green, with flowerheads.
December, 1933.	09	5.0	4.6	1.10	1.6	0.36	1.5	1.98	40.1	1280.0	Mixed, mainly green, with flowerheads.
January, 1934.	14	5.7	3.5	1.10	1.9	0.18	1.6	2.03	34.0	2860.0	Green, seeds falling out.
February, 1934.	10	4.5	2.6	9.4	1.4	0.14	1.3	1.89	43.1	3200.0	Mixed, mainly green, seeds falling out.
FASPALUM DILATATUM.											
September, 1933	10	4.6	5.9	9.9	4.2	0.32	1.7	2.78	32.6	310.0	Mixed, mainly brown, seeds falling out.
October, 1933.	07	4.7	5.8	6.5	3.2	0.27	0.8	2.83	35.3	507.0	Mixed, mainly brown, seeds falling out.
November, 1933	22	10.7	5.3	2.08	4.1	0.53	2.6	4.58	32.7	420.0	Mixed, mainly green, few flowerheads.
December, 1933	25	7.6	3.6	2.59	4.0	0.54	3.1	4.52	36.9	570.0	Green, with flowerheads.
January, 1934.	16	6.0	2.7	2.53	3.1	0.60	4.0	3.40	36.3	1300.0	Green, seeds falling out.
February, 1934	10	4.0	2.1	2.85	2.8	0.83	4.9	4.34	43.9	2750.0	Mixed, mainly green, seeds falling out.
ERAGROSTIS PLANA.											
September, 1933	12	6.0	4.6	4.5	1.4	0.08	1.4	1.65	35.1	115.0	Mixed, mainly brown, seeds falling out.
October, 1933.	10	6.2	3.7	3.4	1.5	0.18	0.8	1.61	39.4	105.0	Brown, seeds fallen out.
November, 1933	19	10.0	4.3	1.75	1.9	0.47	2.6	3.05	41.3	540.0	Mixed, mainly green, flowerheads.
December, 1933	16	6.6	3.6	1.65	2.8	0.54	3.0	2.74	43.7	960.0	Mixed, mainly green, with flowerheads.
January, 1934.	13	4.2	2.8	1.46	1.0	0.15	2.6	2.21	43.1	2120.0	Green, with seeds.
February, 1934	—	—	—	—	—	—	—	—	—	2950.0	Mixed, mainly green, seeds falling out.

MINERAL CONTENT AND FEEDING VALUE OF NATURAL PASTURES.

TABLE M. (continued).

Date of cutting.	P <sub>2</sub> O <sub>5</sub>	Crude Protein.	CaO.	K <sub>2</sub> O.	MgO.	N <sub>2</sub> O.	Cl	Soluble ash.	Crude fibre.	Total yield in grams	Description of cuts.
CHLOERIS GAYANA.											
September, 1933.	.11	4.5	.58	.34	.32	.78	.87	2.97	32.6	530.0	Mixed, mainly brown, seeds fallen out.
October, 1933.	.06	4.2	.55	.25	.25	.57	.55	2.63	42.5	560.0	Brown, seeds fallen out.
November, 1933.	.18	8.6	.59	1.48	.31	.80	.78	4.48	34.8	700.0	Mixed, mainly green, few flowerheads.
December, 1933.	.11	5.4	.58	1.29	.27	.62	.88	4.16	39.5	580.0	Mixed, mainly green, with flowerheads.
January, 1934.	.08	4.1	.42	1.21	.27	.58	.68	3.34	37.2	3000.0	Green, seeds falling out.
February, 1934.	.10	4.9	.49	.92	.29	.57	.75	3.48	36.1	6700.0	Mixed, mainly green, seeds falling out.
ALLOTROPIS SEMIALATA.											
September, 1933.	.22	6.6	.55	.65	.28	.019	.14	2.13	43.4	45.0	Mixed, seeds fallen out.
October, 1933.	.22	8.3	.47	.99	.27	.016	.42	2.80	49.4	108.0	Mixed, mainly green.
November, 1933.	.39	13.1	.34	2.78	.48	.063	.31	4.31	42.3	240.0	Green, few flowerheads.
December, 1933.	.31	7.6	.20	2.22	.27	.062	.38	3.07	41.7	570.0	Green, with flowerheads.
January, 1934.	.24	5.7	.18	2.05	.23	.026	.21	2.82	44.5	840.0	Green, with seeds.
February, 1934.	—	8.3	.29	1.93	.27	.027	.23	3.25	46.0	860.0	Green, seeds falling out.
ELYONURUS ARGENTENS.											
September, 1933.	.18	6.8	.69	.81	.41	.016	.14	2.49	26.2	210.0	Mixed, seeds falling out.
October, 1933.	.13	6.1	.53	.88	.27	.016	.25	2.95	32.2	230.0	Mixed, few flowerheads.
November, 1933.	.18	8.5	.41	1.17	.33	.053	.24	2.80	34.9	550.0	Mixed, mainly green, few flowerheads.
December, 1933.	.23	7.3	.46	1.67	.25	.039	.23	3.20	36.7	480.0	Green, with flowerheads.
January, 1934.	.14	6.4	.36	1.12	.26	.013	.16	2.43	36.8	520.0	Mixed, mainly green, seeds fallen out.
February, 1934.	.13	4.9	.35	.82	.22	.019	.09	1.91	43.9	1040.0	

## Section VIII.

# Surgery.

QUINLAN, J., DE KOCK, G., AND MARAIS, I. P. The operation of splenectomy in horses, cattle, sheep, goats, pigs, dogs and some S.A. antelopes. A summary of the results of 98 splenectomies.





## **The Operation of Splenectomy in Horses, Cattle, Sheep, Goats, Pigs, Dogs, and some South African Antelopes: A Summary of the Results of 98 Splenectomies.**

By

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and  
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WITHIN recent years the advance in our knowledge of the functions of the spleen and its rôle in certain diseases of the blood have justified the operation of splenectomy as a recognised treatment in certain forms of anaemia in human medicine; removal of the spleen is no longer confined to cases of splenic trauma.

In the lower animals the operation of splenectomy, as a curative measure, has been undertaken only in the dog in the case of traumatic injury and in splenic newgrowths. There is little doubt that the use of the operation can be successfully extended and will be in the future, with the progress of surgery in the lower animals. The operation would save the lives of dogs that suffer from spontaneous splenic rupture, which sometimes occurs as a sequel to canine piroplasmiasis. However, splenectomy in canine medicine is confined to veterinary specialists and has not yet become a general practice.

The operation of splenectomy in veterinary medicine has been mostly of an experimental nature. For this purpose it has been carried out on dogs in many countries. De Kock and Quinlan (1926) operated successfully on horses, cattle, sheep, and goats. The literature appears scanty in reference to the operation except in the dog. Warthin (1903) published the results of splenectomy in sheep and goats. His results were not satisfactory, as 50 per cent. of the animals operated upon died from shock. In this connection Warthin's results are surprising, as shock following the operation has been a rare occurrence in over ninety splenectomies performed by one of us (Q) in the conduction of a series of experiments on piroplasmiasis, theileriosis, eperythrozooniasis, anaplasmosis, and trypanosomiasis.

Removal of the spleen was first introduced at this Institution to study the nature of the reticulo-endothelial system, in which one of us (de K.) was interested. It was begun on horses and was followed by such important results that it was extended to cattle, sheep, goats, and antelopes with equally important sequelae (de Kock and Quinlan, 1926).

Since the publication of the abovementioned work the use of the operation has been extended at this Institution as a means of rendering animals susceptible to certain protozoal diseases and also to demonstrate whether they were carriers of infection. For this purpose it is highly successful.

The operation has been applied in the study of the life cycle of certain blood parasites. It has been used in the diagnosis and the study of susceptibility and immunity to diseases such as anaplasmosis, piroplasmosis, trypanosomiasis, and eperythrozooniasis. Further the value of splenectomy in the study of the destruction of erythrocytes, the metabolism of the pigment of the blood, and the reticulo-endothelial system has been appreciated.

The results of completed studies have already been published in the Reports of the Director of Veterinary Services and Animal Industry and in the Onderstepoort Journal. The experimental work now in progress will be published later in the Onderstepoort Journal.

Splenectomy has been successfully performed on horses, cattle, sheep, goats, pigs, dogs, and a few South African antelopes, viz.: the blesbok (*Damaliscus albifrons*) and the duiker (*Sylvicapra grimmii* L.).

Since de Kock and Quinlan (1926) published their paper some other workers, using almost similar operative technique, have splenectomized bovines (Meissner, 1931, Rees, 1933). These are the only references that can be traced in the literature to the operation in the larger domestic animals. However, it appears likely, now that the operation has been performed extensively and successfully, that it will facilitate the task of research workers in tropical and sub-tropical countries.

In this paper it is not intended to recapitulate in detail the results of removal of the spleen in the lower animals as already published by de Kock and Quinlan (1926, 1927), de Kock (1929), du Toit (1931), Meissner (1931), Rees (1933), Neitz and du Toit (1932). The object of the paper is to give an account of the operative technique and the methods of anaesthesia used, as well as a summary of the result obtained in animals splenectomised subsequent to the first publication of de Kock and Quinlan (1926).

## THE OPERATION.

### GENERAL REMARKS.

In the case of equines it is advisable to select young and small animals as the operation is very difficult even in medium-sized horses. The first operation performed was on an adult mare of small size with a wide open "coupling". The operation was successful, but it was

extremely tedious and tiresome and, as will afterwards be pointed out in the paragraph on operative technique, the danger of peritonitis is very real, because of the protracted manipulations which are necessary within the abdomen. After this first experience immature animals were used, when selection was possible, that is horses under two years and cattle under eighteen months. In one case an ox weighing seven hundred pounds was operated upon. Such an operation requires considerable physical strength as well as technical skill and should not be undertaken by surgeons with a fragile constitution. The best surgical results can be obtained with very small animals as the operation can be carried out expeditiously and short abdominal manipulation only is necessary.

The subjects, where selection is possible, should be in good hard condition. Obesity is undesirable, while very poor animals may show shock. In the case of the smaller animals such as sheep, goats, pigs, and dogs obesity is objectionable as the subcutaneous and intra-abdominal fat somewhat impede laparotomy. This, however, is the only objection.

#### A. THE OPERATION IN HORSES.

##### *Preparation.*

The following precautions are taken before bringing the animals into the operating theatre. The preparation includes dieting, purging, and starving with the object of reducing as far as possible the volume of the stomach and intestines. The horses are put on a concentrated non-bulky diet eight days prior to the operation. The diet consists of oats and bran with a few pounds of green lucerne daily. Water is given *ad libidum*. Food and water are withheld and a mild purgative administered 36 hours before the operation. The horses are clipped and washed the day previous to operating and a large area over the left flank shaved and painted with tincture of iodine. From this time sterilized bedding only is used in the box.

##### *Anaesthesia.*

General anaesthesia is adopted. Chloral hydrate in a solution of 10 per cent. with normal saline is used in company with chloroform inhalation. The dose given varies from  $2\frac{1}{2}$  to 3 grams per 100 pounds body-weight. It is given intravenously at the rate of 50-75 c.c. per minute. Deep anaesthesia is produced by chloroform inhalation following the injection of chloral hydrate. The chloroform is administered through an open mask.

This method of anaesthesia for equines has been used at this Institution for a number of years with complete success. In no case was there the slightest cause for anxiety as to the welfare of the patient during the operation. The horses recovered rapidly from the effects of the anaesthetic and did not show symptoms of operative shock.

##### *Technique.*

The patient is placed on its right side on the operating table. The fore limbs and head are fixed in the most convenient position, while the hind limbs, with the joints extended, are drawn slightly backwards so as to make the region of the flank as approachable as possible.

The skin over the shaven area in the left flank is then liberally swabbed with ether. The area surrounding the field of operation is covered with sterile linen which is held in position by cloth forceps. The hands and arms, after a thorough cleansing with synol soap and warm water, are bathed with alcohol-corrosive sublimate solution (1—1,000). They are finally rinsed in sterile physiological saline.

The instruments, after sterilisation in the autoclave, are used dry or transferred to sterile physiological saline.

### *Laparotomy.*

A vertical incision is made through the skin and muscles down to the peritoneum in the region of the left flank about five centimetres behind the last rib. The incision is begun as close to the transverse processes of the lumbar vertebrae as possible, as this position facilitates the approach to the splenic vessels when the remainder of the organ has been mobilised. The length of the incision varies from 15 cm. to 25 cm. according to whether the patient is a small or large animal. In large animals the longer incision is necessary as both hands and arms must be used inside the abdomen at the same time. Haemorrhage is now arrested. The peritoneum is caught up in a forceps and incised in the direction of the cutaneous incision for a distance of about half an inch.

A large piece of sterile gauze is placed over the field of operation and an incision made through it over the length of the wound. The gauze is attached to the lips of the wound by sutures or forceps. Sutures are preferable as they do not interfere with later manipulations. The peritoneum is included in the sutures. It is gradually opened as the suturing extends towards the extremities of the incision. The suture material is not cut off short but is looped so that it may be used for dilating the wound when necessary.

When mobilising the spleen the relations of the intra-abdominal organs are disturbed as little as possible. To maintain the normal relationship it is necessary to leave the cavity of the omentum intact by laying on a line of sutures which will unite the gastro-splenic and the greater omentum.

The mobilisation of the spleen differs somewhat in small and medium-sized animals. In the latter only a small portion of the apex of the spleen can be withdrawn, so that all the ligatures have to be completed within the abdomen. In consequence both hands must be used for intra-abdominal manipulation. Using fine silk for ligaturing, operations are begun at the apex of the spleen. The gastro-splenic and greater omentum are caught between the thumb and fingers of the left hand, while the right hand carrying a half-circle needle and ligature silk is passed over the parietal surface of the spleen. The needle is passed from before backwards through the omentum, and then, having isolated a portion it is again passed from back to front and withdrawn through the laparotomy opening. The ligature is completed by knotting the silk. For the purpose of applying tension and also to facilitate knotting a short piece of plated steel, one-and-a-half inches long, is attached to the free end of the ligature silk. By this means the fingers of the left hand working inside the abdomen can easily apply the knot. Proceeding in this way a double

row of sutures is laid on uniting the gastro-splenic and the greater omentum along the entire length from the apex to the hilus of the spleen until the splenic vessels are reached. The attachment is divided between the double row of ligatures by means of a long blunt-pointed scissors. The suspensory ligament is then treated in a similar manner until the spleen is attached only by the splenic vessels and nerves. A last double ligature is passed around the vessels and nerves at the hilus. The spleen is then detached with the scissors close to the distal ligature and withdrawn.

In ligating, the close connection of the stomach with the spleen must be remembered and the organ protected from injury. While ligating the splenic vessels care must be exercised that too much tension is not applied as the splenic vein may rupture and cause considerable haemorrhage. To obviate this accident the vessels are first clamped with a long forceps and then ligatured. This method facilitates the application of a reinforcing ligature for extra safety after the spleen has been removed.

The abdominal incision is then closed in three layers without drainage. Chromatised catgut is used for the peritoneum and the muscles and silk for the skin. At first two tape tension sutures as well as as small gauze drain were used, but they appear to be unnecessary and have a disadvantage in that there is more likelihood of suppuration. The wound is sealed with collodion and iodoform and covered with gauze and mastisol.

The operation is completed by the application of a sterile protective bandage.

In small horses the operation is not so difficult as a large portion of the spleen can be withdrawn through the abdominal incision, and the application of many of the ligatures can be done under direct observation. An incision 15 cm. in length is sufficient, since it is necessary to introduce only one hand into the abdominal cavity. In foals the apex of the spleen can be withdrawn and most of the omental ligatures as well as those on the suspensory ligament can be applied outside the abdominal cavity. In this way the organ can be considerably mobilised. Then the left hand is introduced and the remaining attachments—small portion of the gastro-splenic and great omentum and the suspensory ligament surrounding the splenic vessels—are pressed together and caught in a strong forceps. A silk ligature is applied on the compressed tissues and the spleen removed with the scissors.

Outside this small technical change the operation in both small and large horses is identical.

### *Difficulties.*

These are chiefly concerned with the application of the ligatures in large horses, as the intra-abdominal manipulations are not under direct observation. The mobilisation of the organ should be completed as far as possible before attempting withdrawal, as it very easily ruptures and causes unpleasant haemorrhage. The operation causes considerable fatigue on account of the slight elasticity of the thoracic wall and the position which necessarily has to be assumed by the operator.



Fig. 1.—Equine 16072. 2 weeks after operation



Fig. 2. —Equine 16072, 5 weeks after operation

## B. THE OPERATION IN CATTLE.

The preparation, anaesthesia, and the laparotomy are similar to what has been described for horses. A slight modification is, however, necessary with the position of the cutaneous incision, which is made parallel to the last rib and about 5 cm. posterior to it. It is begun as high up as possible in the angle between the posterior rib and the transverse processes of the lumbar vertebrae; a length of 15 to 20 cm. is sufficient.

The left hand is introduced into the abdomen between the rumen and the visceral surface of the spleen. Beginning at the antero-inferior aspect of the area of attachment the peritoneum is perforated along the line of its reflection from the rumen to the spleen and the connective tissue broken down by careful blunt dissection. When difficulty is experienced in breaking through the tissue with the fingers, as is sometimes the case in adult animals, it is cut through with a long-handled scissors. Proceeding by means of blunt dissection the detachment is completed over the face of the rumen and the adjacent face of the reticulum, as well as over the left crus of the diaphragm, until it remains attached only by the splenic vessels and nerves at the hilus. The spleen is then withdrawn and the vessels caught in a strong forceps. A double ligature is placed on the vessels about 2 to 3 cm. from the hilus and the spleen is detached by cutting between the ligatures with a scissors. The forceps is opened slowly to observe whether the ligature has completely occluded the vessels before releasing the stump.

Intra-abdominal manipulation should be gentle and careful as rupture of the capsule of the spleen will cause profuse and troublesome haemorrhage.

The abdominal wound is closed by suturing the peritoneum and muscles with catgut and the skin with silk. Post-operative protection of the wound is carried out as in equines.

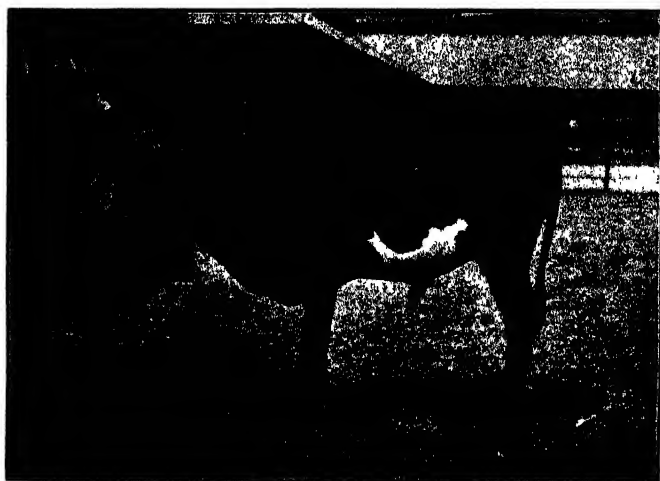


Fig. 3.—Bovine 894, 3 weeks after operation.



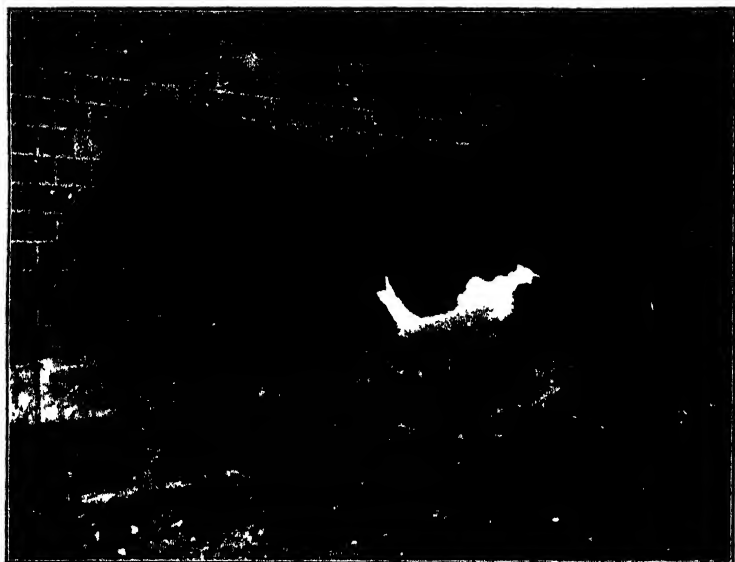


Fig. 4.—Bovine 894, 7½ months after operation.



Fig. 5.—Bovine 894, 2 years after operation.



Fig. 6.—Bovine 893, 1 month after operation.

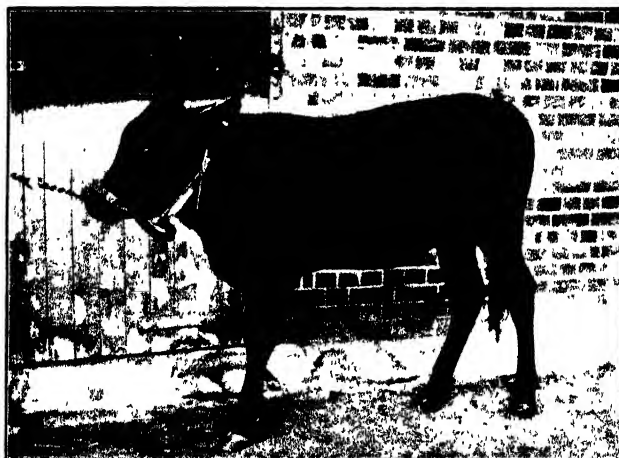
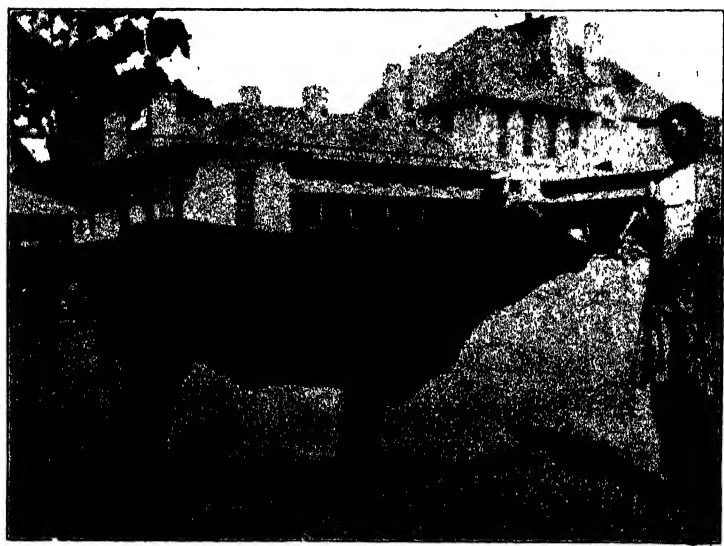


Fig. 7.—Bovine 893, 5 months after operation.

**SPLENECTOMY IN DOMESTIC ANIMALS AND SOME S.A. ANTELOPES.**



**Fig. 8.—Bovine 893, 2 years and 4 months after operation.**



**Fig. 9. Bovine 2611, 10 months after operation.**

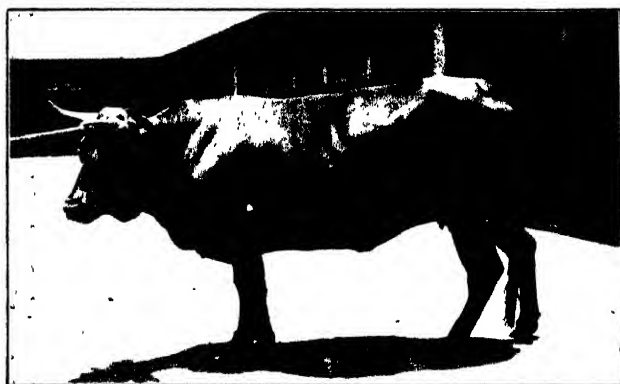


Fig. 10.-Bovine 2611, 3½ years after operation.

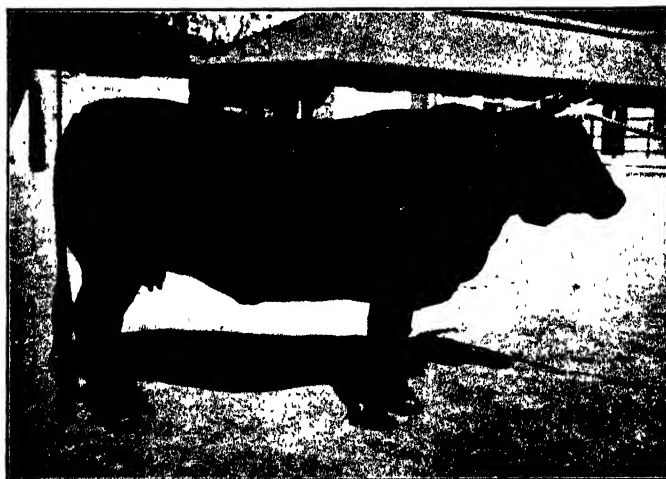


Fig. 11. -Bovine 2611, 1 years and 2 months after operation.

## C. THE OPERATION IN THE SHEEP, GOAT, AND ANTELOPE.

The technique of the operation in the sheep, goat, and antelope differs little from that described in bovines. The oral administration of chloral hydrate for anaesthesia, which was used in the commencement, was later superseded by intravenous injection. The laparotomy is done in the same situation. The left hand is inserted into the abdomen and passed over the parietal surface of the spleen. The attachment between this surface and the diaphragm is first broken down by blunt dissection. Then beginning at the most inferior portion of its attachment to the dorsal curvature of the rumen the tissue is broken down up to the hilus. So as to prevent stripping of the peritoneum from the surface of the rumen a long curved scissors is used as an aid to blunt dissection with the fingers. Small portions of peritoneum and fibrous tissues are isolated and cut through. The remaining attachment to the left crus of the diaphragm is broken down in a similar manner proceeding cranially. The detachment of the spleen is carried out in a similar way to that described in the bovine. The wound in the abdominal wall is also closed in a similar manner. Post-operative protection is carried out as in equines.

The capsule of the spleen in sheep and goats is very easily ruptured. If such an accident occurs there is profuse haemorrhage, consequently dissection of the splenic attachments must be carried out with gentleness and patience.



Fig. 12.—Ovines 28498 and 28578, nine months after splenectomy.



Fig. 13. Group of splenectomised sheep, one of which, No. 8429 had been splenectomised 6 years 7 months previously.

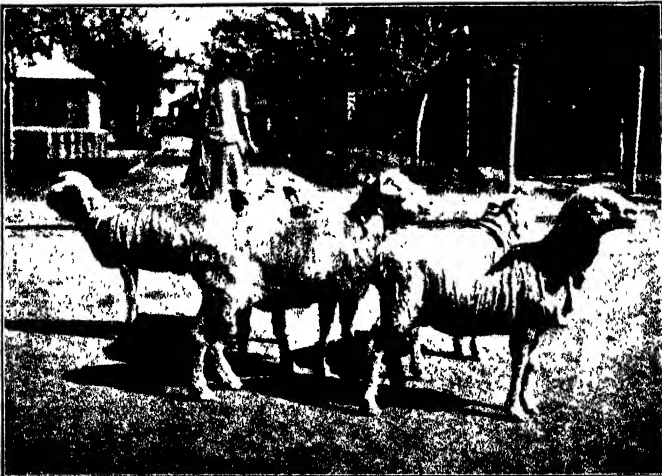


Fig. 14.—Group of splenectomised sheep, one of which, No. 16023. had been splenectomised 6 years 5 months previously.

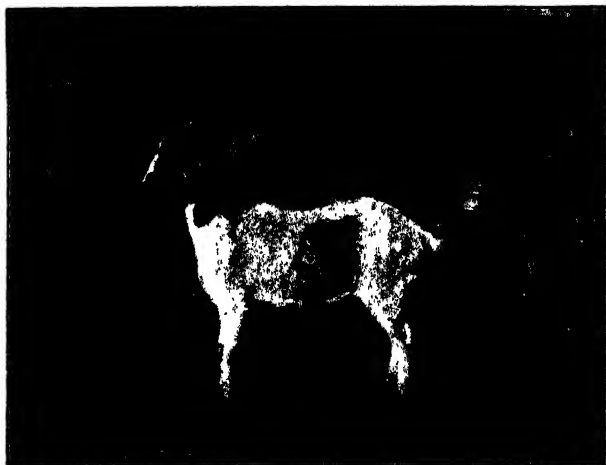


Fig. 15.—Caprine 8304, 3 weeks after operation.



Fig. 16.—Caprine 8280, 3 weeks after operation.

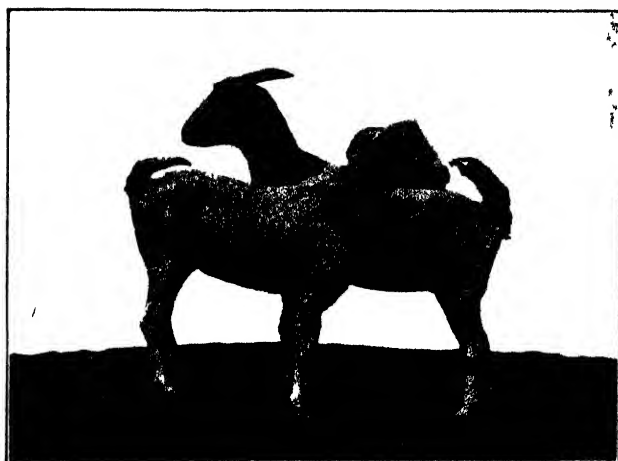


Fig 17 Caprines 8280 and 8304 7½ months after operation

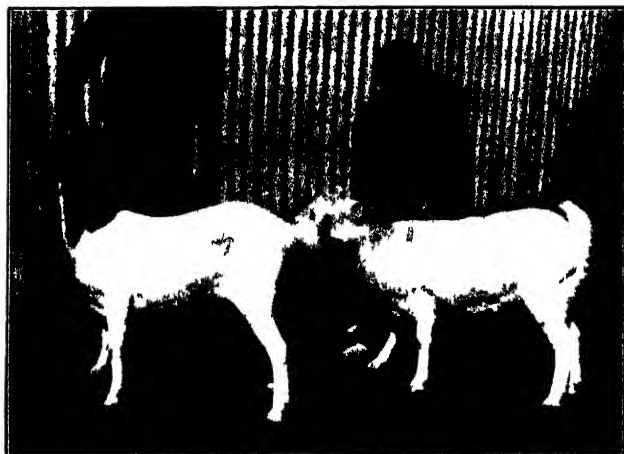


Fig 18 —Caprines 8280 and 8304, 2 years and 10 months after operation



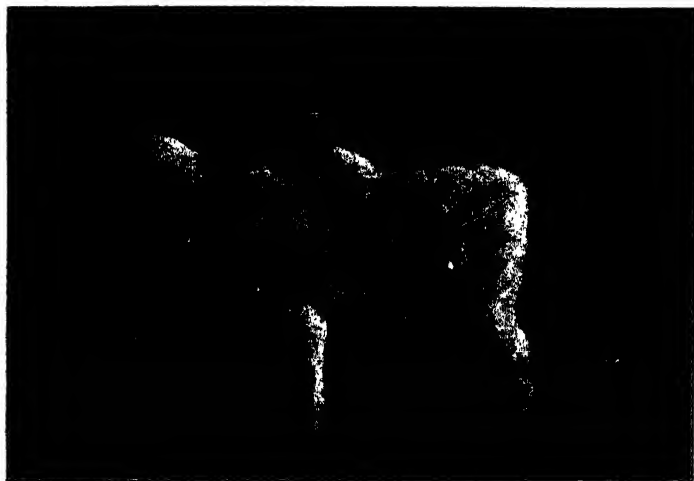


Fig. 19.—Caprine 8280, 6 years 11 months after operation.



Fig. 20.—Duiker (*Sylvicapra grimmia grimmia* L.) 32806, one day after operation.

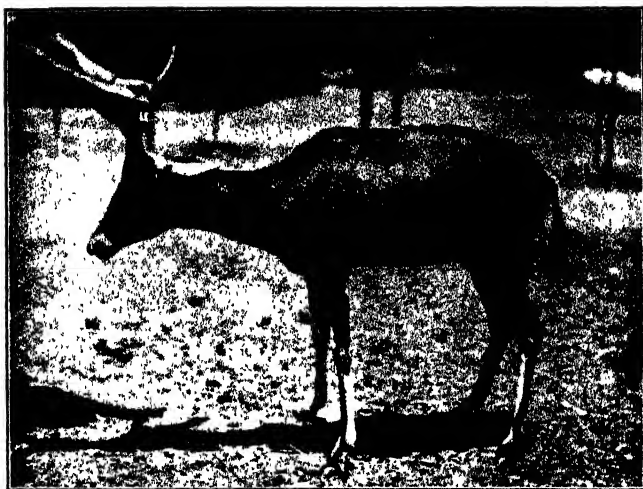


Fig. 21.—Blesbuck (*Damaliscus albifrons*) 33606, 8½ months after operation.

#### D. THE OPERATION IN THE PIG.

The pig is also carefully prepared for the operation. Pernocton, by the intravenous route, using one of the auricular veins, is used as an anaesthetic. This method of anaesthesia for the pig is excellent. The dose used is 0.03 grams per kilogram body-weight.

The route of approach to the spleen is immediately behind the last rib on the left side, beginning as close to the lumbar transverse processes as possible. The incision runs parallel to the rib. A short incision, about 8 cm., is sufficient as the spleen can be easily and completely withdrawn through such a small opening. One or two fingers are introduced into the abdomen and the spleen drawn through the opening. A double series of fine silk ligatures are then placed along the splenic attachment at the hilus and the organ detached by cutting between the ligatures with the scissors. The abdominal wound is closed by three series of sutures; those for the peritoneum and the muscles being of catgut and those for the skin silk. Post-operative protection of the sutured wound other than the usual seal is unnecessary.

#### E. THE OPERATION IN THE DOG.

There is no difference between the technique of the operation in the dog and that described for the pig except that the spleen can be withdrawn either through a mid-ventral or a low flank incision. Of the two routes of approach the flank incision is preferred and will be used in future operations.

Anaesthesia is accomplished in canines by the use of subcutaneous eukodal followed one hour later by intravenous pernocton; 1 c.c. of eukodal is used for each 5 kilograms body-weight and 0.03 grams of pernocton per kilogram body-weight. The previous injection of eukodal frequently reduces the amount of pernocton necessary for deep and prolonged anaesthesia.

SPLENECTOMY IN DOMESTIC ANIMALS AND SOME S.A. ANTELOPES.



Fig. 22.—Porcines 903, 904, 905 and 906, 16 days after operation.



Fig. 23.—Porcine 904, 10 months after operation.



Fig. 24.—Porcine 905, 10 months after operation.



Fig. 25.—Canine 1073, 3½ months after operation.



Fig. 26.—Canines 1044, 1143, 8½ months after operation.

#### AFTER-TREATMENT.

After the operation the animals are placed in boxes in which sterilised bedding is used. It appears to be a useful precaution, especially for equines in which the tendency to wound infection appears to be far greater than in the other species of animals operated upon. The precaution was not taken entirely with a view to obtaining healing of the wound *per primam intentionem*, but also to ensure against the possibility of tick infection, which would have rendered the experiments which were being conducted with tick-borne diseases unsatisfactory.

The food following operation consisted of a light laxative ration, composed chiefly of small quantities of crushed oats with bran and green lucerne for horses, and maize meal with bran and green lucerne for cattle, sheep, goats, and antelopes. Water was allowed *ad libidum*.

Dogs and pigs were fed on milk and maize meal porridge, with the addition of beef-extract in the case of dogs.

The wounds were inspected daily and the dressings renewed. In the case of cattle, sheep, goats, pigs, and dogs there was no trouble with the wounds. They invariably healed *per primam intentionem*. The cutaneous sutures were removed on the seventh or eighth day following the operation.

In the case of equines the wounds did not heal by first intention. Probably the difficulty did not lie in the wound itself but rather in the nature of the disease which occurred as a sequel to the removal of the spleen, where changes in the blood were sufficient to prevent healing. Even in those cases the incisions in the peritoneum and the internal oblique muscle had united, but there was no adhesion between the incised lips of the external oblique muscle or the skin. These structures were healing under granulation when the animals died of the concurrent disease. In one horse which was free from *Nuttallia equi* infection, and which recovered, the upper two-thirds of the wound healed by first intention but the skin over the lower

third suppurated. However, this portion of the wound healed nicely under granulation. The resulting cicatrix was scarcely visible when the animal died four months later as a result of an intravenous injection of haemolytic serum.

## SUMMARY OF THE RESULTS OF SPLENECTOMY.

De Kock and Quinlan published the results of their first series of experiments on splenectomised horses, cattle, sheep, and goats in 1926. Since then observations have been continued and extended to other species of domesticated animals as well as to wild antelopes. The results of the later splenectomies have been published mostly as isolated papers in various subsequent Reports of the Director of Veterinary Services. (De Kock and Quinlan, 1927; de Kock, 1929; du Toit, 1931; Neitz and du Toit, 1932.)

### *Horses.*

Of the four horses splenectomised three were immune "carriers" of *Nuttallia equi* and all three showed fatal relapses of Nuttalliosis in from 3 to 8 days after the operation. The fourth animal was a young foal, not a carrier of Nuttalliosis. It was kept under tick-free conditions for four months following the operation when it was killed by injections of haemolytic serum.

### *Cattle.*

Five animals were observed by de Kock and Quinlan (1926 report) and their findings may be summarised briefly as follows:—

(1) Relapses of Theileriosis (*Theileria mutans*), anaplasmosis, and piroplasmosis (*P. bigeminum*) occurred in immune "carriers" of these parasites. Piroplasmosis relapses were in these cases particularly severe.

(2) These diseases in splenectomised animals assumed a chronic course particularly with regard to the blood changes.

A total of 23 bovines were splenectomised of which 20 recovered from the operation. One animal died three days afterwards, and on post mortem no apparent cause of death could be established; one died as a result of haemorrhage and one from surgical shock.

Some of the animals were used in East Coast fever transmission experiments and two animals which had recovered from East Coast fever were splenectomised in order to try and break down their immunity (du Toit, 1931). Removal of the spleen was found to have no effect on the course of this disease or on the immunity.

Two bovines were splenectomised for further study of the blood changes in anaplasmosis. One animal, No. 4627, infected with the mild *A. centrale* strain, reacted so severely after splenectomy that it died as the result of the extensive blood changes.

Two calves, Nos. 4658 and 4676, susceptible to anaplasmosis and piroplasmosis, were injected with blood from sheep showing *anaplasma ovis* and then splenectomised to see whether the *anaplasma ovis* would appear in the calves' blood, or whether the calves could

become "carriers" of the disease. After the operation, however, both animals became accidentally infected with bovine piroplasmosis and anaplasmosis and sheep sub-inoculated from them showed no reaction.

### *Sheep.*

Fifty-four sheep have been splenectomised of which seven died as the direct result of the operation. In one of the latter, namely No. 8462, the attachments of the spleen were loosened and the splenic vessels ligated, but the organ itself left *in situ*.

The most striking result of de Kock and Quinlan's observations on sheep was the discovery of the hitherto unsuspected presence of an anaplasma, *A. ovis*, and a Theileria, *T. recondita* (called *Gonderia ovis* by them) present in the blood of some South African sheep.

*Anaplasmosis of Sheep.*—The result of their observations can be briefly summarised as follows:—

- (1) Non-splenectomised susceptible sheep can be infected by means of blood inoculation. The disease then runs a mild course with distinct anaemic changes and the appearance of parasites in the blood in large numbers, but no mortality.
- (2) In splenectomised "carriers" and splenectomised susceptible sheep the course is much more severe and protracted and there is a fairly high mortality.
- (3) The disease could not be transmitted to splenectomised or non-splenectomised bovines nor did these animals become carriers of the parasites.
- (4) Non-splenectomised goats could not be infected but in splenectomised goats parasites appeared in the blood with very slight anaemic changes, and such animals became carriers of the disease.

Sheep, splenectomised and non-splenectomised, did not react to bovine anaplasmosis nor did they become carriers of the parasites.

*Theileriosis of Sheep.*—De Kock and Quinlan (1927) reported on the occurrence of Theileria (*T. recondita*, *Gonderia ovis*) in the blood of some splenectomised sheep. Transmission to non-splenectomised sheep and to bovines failed. The parasites did not produce any symptoms and appeared to be quite harmless.

*Histological Changes.*—De Kock (1929) reported on the appearance of haemo-lymphoid-like nodules in the liver of sheep, bovines and a goat killed three years after splenectomy. The nodules varied in size from  $\frac{1}{2}$  in. to  $1\frac{1}{2}$  in. in diameter, were dark red in colour, and circumscribed. In some instances a distinct capsule could be recognised, and in others there was no demarcation between this foreign tissue and the liver substance. Lymphoid nodules could be recognised in some of these structures. The haemo-lymphoid-like nodules were not seen in the animals which died or were killed within the first two years following splenectomy. The first indications being found in a sheep which died 33 months after the spleen was removed. From the microscopic appearances de Kock concludes that this newly formed tissue resembles most closely that of haemolymph glands.

### Goats.

Before splenectomy the two animals were injected with blood from sheep showing *anaplasma* but no reaction followed nor could *anaplasma* be demonstrated. Splenectomy failed to produce a relapse as in the case of immune carriers. Subsequent to the operation, however, when reinjected the blood showed the presence of *anaplasma* with practically no anaemic changes.

The histological changes which were found at autopsy on the one goat which died 8½ years later have been mentioned above.

### Canines.

Seven dogs were successfully splenectomised. One animal showed the presence of *microfilaria* in the blood three days later and continued to show these parasites until it died from other causes. In no case did *piroplasma* appear in the blood as the result of removal of the spleen. However, it was only assumed that the animals were carriers of *Piroplasma canis* as they had been exposed to tick infestation, but there can be no certainty with regard to this. Subsequently three of these dogs were injected with blood from a known carrier of the disease. One of them died from piroplasmosis and two recovered after treatment, but continued to show parasites for several weeks afterwards.

Owing to the uncertainty with regard to the previous immunity it is not possible to draw definite conclusions on the effect of the removal of the spleen on canine piroplasmosis. The disease, however, appeared to affect the splenectomised dogs more severely than is experienced in clinical cases of the disease in normal dogs. One of the animals showed a severe haemoglobinuria. Miessner (1931) reports on a few cases of relapses of piroplasmosis following splenectomy in dogs. The experiment will be repeated with known carriers of *Piroplasma canis*.

Four dogs were injected with the virus of African horsesickness after splenectomy but did not show any reaction to the disease.

### Porcines.

Four pigs were splenectomised and all made uneventful recoveries. No parasites appeared in the blood following the operation. Attempts were made to infect the splenectomised pigs with African horsesickness (two cases) and with ovine blue tongue (two cases) without success.

### Antelopes.

Neitz and du Toit (1932) reported on a method of obtaining pure strains of *Anaplasma marginale* and *centrale* by transmission through antelopes. The antelopes were injected with blood from bovine carriers of *Piroplasma bigeminum*, *Theileria mutans*, and either *Anaplasma marginale* or *A. centrale*. In each case only the *anaplasma* appeared in the blood. These antelopes were then splenectomised in order to exclude the possibility of the other parasites from the donor's blood being present in a latent form. In the cases of the two that survived the operation only the *anaplasma* reappeared in the blood after splenectomy. Three blesbuck (*Damaliscus albifrons*) and one grey duiker (*Sylvicapra grimmii grimmii* L.) were operated on.



One blesbuck died soon after the operation and the other two recovered. The duiker seemed to recover completely but interfered with the wound on the eighth day and died as a result.

One of the blesbuck, No. 33606, was later injected with the virus of ovine blue tongue and although it showed no reaction it was found to be a carrier on subinoculation of susceptible sheep.

*Spirochaeta theileri* could also be transmitted to the blesbuck.

*Heartwater*.—Finally the animal was injected with heartwater from a sheep and died 23 days later of this disease. *Rickettsia* could be demonstrated and susceptible sheep were infected from it.

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Table I shows details of all the animals splenectomised and their subsequent history.

TABLE I.

Species.	Number	Weight. lb.	Date of operation.	Anaesthesia.		Duration.	Weight of spleen.	Result	Experi- ment.	Subsequent History.
				Chloral Hydrate.	Chloroform.					
Equine.....	15186	650	25/10/23	15 gm. intravenously	15 c.c. by inhalation	1½ hours	2.70 Kg.	Recovered	S. 1602	Killed in extremis, 31/10/23. Recurrence of nutalliosis.
" .....	15420	680	21/11/23	18 gm.	45 c.c.	1½ hours	3.40 Kg.	"	"	Died 24/11/23. Recurrence of nutalliosis.
" .....	16032	400	23/10/24	10 gm.	45 c.c.	2½ hours	2.10 Kg.	"	"	Killed in extremis, 31/10/24. Recurrence of nutalliosis.
" .....	16072	320	7/3/24	6 gm.	60 c.c.	4 hours	2.00 Kg.	"	"	Died 13/6/24 from effect of injection of anaplasma.
Bovine. ....	711	380	27/3/24	10 gm.	120 c.c.	3 hours	1.05 Kg.	"	"	Recurrence of <i>anaplasma centrale</i> in blood. Animal recovered.
" .....	758	325	6/8/24	Few drops	"	1½ hours	1.70 Kg.	Died Recovered	S. 1602	Injected with blood from sheep showing anaplasma but showed no reaction.
" .....	808	350	20/11/24	12 gm.	60 c.c.	2 hours	2.10 Kg.		"	Injected with blood from bovine showing piroplasmiasis, 14/3/25.
" .....	893	350	16/12/24	15 gm.	80 c.c.	1½ hours	3.10 Kg.		S. 1983	Injected with blood from animal killed from piroplasmiasis 26/6/24.
" .....	1029	390	22/12/24	15 gm.	30 c.c.	1½ hours	?	Died	S. 1602	Died 23/11/24. Unknown cause.
" .....	1027	425	24/12/24	15 gm.	60 c.c.	1½ hours	1.85 Kg.	Recovered	"	Showed recurrence piroplasmiasis, theileriosis (mutans), was treated and recovered.
" .....	1034	397	20/1/25	15 gm.	30 c.c.	2 hours	1.75 Kg.	"	S. 2117 etc.	Infected with east coast fever, recovered and was used in various east coast fever experiments.
" .....	894	375	17/3/25	12 gm.	50 c.c.	2½ hours	?	"	S. 1602	Died trypanosomiasis, 7/10/27.
" .....	1032	450	21/4/25	20 gm.	15 c.c.	2 hours	?	"	S. 2117	Died the same day as the result of haemorrhage.
" .....	870	480	30/4/25	16 gm.	15 c.c. ether	2 hours	?	"	"	Showed relapse of piroplasmiasis and theileriosis (mutans), was treated without treatment but continued to show piroplasmiasis in the blood for 3 months.
" .....	3000	320	22/7/29	10 gm.	15 c.c. ether 30 c.c.	1 hour	?	"	S. 1602	Used in east coast fever experiment, 8/7/28 and recovered. Died of piroplasmiasis on being exposed to natural infection, 5/7/28.
" .....								"	S. 3460	East Coast fever transmission and immunity experiments.
" .....								"	S. 2061 etc.	Exposed on tick infected veld, 2/4/28, and died 21/5/28 of piroplasmiasis.
" .....								"	S. 3460	<i>Theileria mutans</i> recovered, died of piroplasmiasis, 20/3/28.
" .....								"	S. 1602	East Coast fever recovered animal and was splenectomised to try and break down the immunity.
" .....								"	S. 2061	Infected with piroplasmiasis and anaplasmosis. Died 12/8/29 of anaplasmosis.

TABLE I (continued).

Species.	Number	Weight. lb.	Date of operation.	Anaesthesia.			Weight of spleen.	Result.	Experi- ment.	Subsequent History.
				Chloral Hydrate.	Chloroform.	Duration.				
Bovine.....	2637	700	9/4/30	35 gm. intravenously	150 c.c. inhalation	4 hours	?	Recovered	S. 3302	Died of anaplasmosis, 25/4/30.
".....	4627	123	17/3/32	7 gm. "	"	1½ hours	?	"	S. 4488	Died of anaplasma sequel to anaplas- mosis, 29/3/32.
".....	4658	218	9/6/32	8 gm. "	80 c.c. "	2½ hours	?	"	S. 4664	Injected with blood from sheep 26331 showing anaplasmosis but did not react. Then splenectomised and showed bovine theileria and ana- plasma. Post-mortem result of acci- dental tick infection and recovered.
".....	4676	186	9/6/32	8 gm. "	30 c.c. "	3 hours	?	Died	S. 4666	Died 20/6/32 apparently of surgical shock.
".....	4688	270	22/6/32	10 gm. "	7.5 c.c. "	2 hours	?	Recovered	S. 5170	<i>Anaplasma centrale</i> carriers. After operation these parasites were recovered and passed on to sheep. Used in further obser- vations of this parasite.
".....	5425	—	6/9/34	18.5 gm. "	75 c.c. "	3 hours	1,700 gm.	Recovered	S. 5170	
".....	5432	400	11/9/34	20 gm. "	75 c.c. "	3 hours	1,487 gm.	Died	S. 5170	
".....	5433	400	20/9/34	20 gm. "	80 c.c. "	3 hours	1,097 gm.	Recovered	S. 5170	
".....	6050	350	14/11/34	9 gm. "	30 c.c. ether "	2 hours	969 gm.	Recovered	S. 5517	Calf was reared as 'tick-free clean calf. 40 days after operation <i>Eperythrozoon venosum</i> appeared. Later splenectomised.
".....	6049	450	14/11/34	11 gm. "	75 c.c. "	3 hours	1,185 gm.	"	S. 5517	Injected with <i>A. centrale</i> and recovered.
".....	6289	180	15, 11 34	7 gm. "	Chloroform. "	1 hour	575 gm.	"	S. 5517	Blood remained negative until 30/1/35. Then used in other experiments.
".....	6295	130	15, 11 34	6 gm. "	7 c.c. "	1½ hours	333 gm.	"	S. 5517	Blood remained negative until 20/2/35. Injected with blood from sheep 41839, carrier of eperythrozoon, and observed in the calf's blood seven days later.
Ovine.....	7369	70	29 '2, 24	12 gm. per os.....	2 c.c. "	3 hours	188 gm	"	S. 1602	Developed <i>anaplasma ovis</i> on 13th day. Killed for post mortem 19/3/34.
".....	7443	74	29.2.24	12 gm. "	2 c.c. "	2½ hours	315 gm.	"	S. 1602	Developed <i>anaplasma ovis</i> on 13th day. Killed for post mortem 22/3/34.
".....	8430	65	8/4 24	8 gm. "	10 c.c. "	1 hour	?	"	S. 1602 S. 1655	Remained normal for 2 weeks. Injected with blood from bovine carrying anaplasmosis with no result.
".....	8431	65	8 4 24	8 gm. "	15 c.c. "	1 hour	?	Died	S. 1825	Injected with blood from sheep showing theileria and <i>anaplasma</i> centralis. Recovered 5 days later. Anaplasma in 7 days. Died after bleeding 30/9/24.
".....	8456	56	15 5 24	8 gm. per os only	—	1½ hours	210 gm.	Recovered	S. 1705	Died from pneumonia, 12/4/24. Injected with blood from sheep showing anaplasma and died 15 days later.
".....	8457	63	15 5 24	8 gm per os.....	Few drops "	1½ hours	194 gm.	"	S. 1705	Injected with blood from sheep showing <i>anaplasma ovis</i> ; showed marked reaction and recovered. Killed for pathological examina- tion, 16/9/24.

TABLE I (continued).

Species.	Number.	Weight. lb.	Date of operation.	Anaesthesia.			Weight of spleen	Result	Experi- ment.	Subsequent History.
				Chloral Hydrate.	Chloroform.	Duration				
Ovine.....	8464	64	15.5.24	8 gm. per os..	6-5 c.c. by inhalation	2 hours	205 gm.	Recovered	S. 1655	Injected with blood from bovine affected with anaplasmosis; no result. Died 21/9.24 of heartwater.
"	8453	64	19.5.24	8 gm.	Drops	1 hour	?	"	S. 1870	Used in <i>T. mutans</i> and anaplasma experiments but failed to react.
"	8454	58	19/5.24	8 gm.	Drops	1 hour	?	"	S. 1999	Subsequently used in black quarter experiments. Died 15/2.26. Cause unknown.
"	8427	58	3.7.24	8 gm.	7-5 c.c.	2 hours	125 gm.	"	S. 1842 S. 1999	Used in various anaplasma experiments but failed to react. Killed 1/4.25 for pathological examination.
"	8428	60	3.7.24	8 gm.	7-5 c.c.	4 hours	184 gm.	"	S. 1613 S. 1602	Before splenectomy was injected with blood of sheep showing <i>anaplasma</i> <i>ovis</i> . Showed anaplasma and recovered. Splenectomised. Developed pneumonia, showed relapse of theileria and died. Killed for pathological examination 18.11.25.
"	8434	56	3.7.24	8 gm.	7-5 c.c.	1 1/2 hours	195 gm.	"	S. 1613 S. 1602	Treated in the same way as above. Showed anaplasma infection before splenectomy and anaplasma and <i>anaplasma</i> <i>ovis</i> after operation.
"	8199 8429	66 58	3.7.24 22.9.24	8 gm. 8 gm.	None, ... by inhalation. Few drops	2 hours	170 gm.	Died Recovered	S. 1613 S. 1602	Treated in same way as above, died of anaplasmosis 16 days after splenectomy.
"	8462	56	22.9.24	6 gm.	Few drops	1 1/2 hours	—	Died	S. 1602	Died during anaesthesia.
"	8455	58	16.1.25	6 gm.	5 c.c.	—	270 gm.	"	—	Treated in same way as No. 8434, but showed only slight reaction both before and after operation.
"	8458	72	16.1.25	8 gm.	Drops	2 hours	220 gm.	"	—	Observed for 7 years afterwards. Spleen not removed but attachments loosened and vessels ligated. Died 23.9.24, three days later.
"	8451	80	28.1.25	8 gm.	Few drops	1 hour	280 gm.	Recovered	S. 1602 S. 2314 S. 2523	Animal collapsed suddenly 5 days after operation and died. Kept under observation for 10 months. Died 3.11.25 of uraemia.
"	9119	45	28.1.25	6 gm.	Few drops	3 hours	70 gm.	"	S. 2523	Kept under observation for 16 months. Injected with bacterial icterus 17/5.26. Died of icterus 20/5.26.
"	10743	68	12.3.25	6 gm.	Few c.c.	1 hour	240 gm.	"	S. 1602 S. 2523	Kept under observation. Injected with cultures producing bacterial icterus, no result.
"									S. 2729	Injected with cultures of case enzootic icterus with no result. Used in Jaagsiekte and other experiments. Died 24.1.31 of toxæmia.

TABLE I (continued).

Species.	Number	Weight. lb.	Date of operation	Anaesthesia.			Weight of spleen.	Result.	Experi- ment.	Subsequent History.
				<i>Chloral Hydrate.</i>	<i>Chloroform.</i>	<i>Duration.</i>				
Ovine.....	10511	75	12/3/25	6 gm. per os.....	Few c.c. by inhalation	1 hour	300 gm.	Recovered	S. 2039	<i>Anaplasma ovis</i> experiment. Kept under observation for 14 months. Injected with culture producing bacterial icterus with no result. Died 7/11/27 of uraemia result of urethral calculus.
"	10944	68	11/5/25	6 gm. intravenously	7½ c.c. "	1 hour	210 gm.	"	S. 2523	Injected with blood harbouring <i>Anaplasma</i> . Then splenectomized 6 weeks later. No relapse occurred.
"	10946	70	11/5/25	6 gm. "	7½ c.c. "	2 hours	300 gm.	"	S. 2069	Used as No. 10944. Died 3rd day after operation from pneumonia.
"	9414	90	6/11/25	5 gm. only	—	1½ hours	?	"	S. 2315	Five days later, 11/11/25, anaesthetized with chloroform and blood samples taken from various organs. Killed with material from a case of enzootic icterus and died 29/6/27 of toxæmia.
"	13952	85	21/2/26	4 gm. "	Few c.c. "	1 hour	?	"	S. 3109	Injected with material from a case of enzootic icterus and died 29/6/27 of toxæmia.
"	13853	70	21/5/26	3½ gm. "	Few c.c. "	1 hour	?	"	S. 2523	Killed for collection of material, 8/6/26.
"	15987	50	12/4/27	3 gm. "	4 c.c. "	1 hour	63 gm.	Died	—	Died of shock 2 days after operation, 14/4/27.
"	16023	50	12/4/27	2½ gm. "	Few drops "	1 hour	81 gm.	Recovered	S. 3051	Injected with blood from sheep, carrier of <i>Anaplasma</i> , and blood, stained chemically and histologically.
"	24355	80	13/7/29	5 gm. "	Ether 10 c.c. by inhalation	1½ hours	?	"	S. 3339	Used for transmission of jaagsiekte.
"	24358	68½	13/7/29	4 gm. "	" "	1 hour	?	"	S. 3599	Kept under observation. Still alive and reserved for further pathological study after death.
"	24356	80	16/7/29	9 c.c. "	" "	1 hour	?	"	S. 3599	Kept under observation. Died 23/9/31 from uraemia, due to urethral calculus.
"	24357	77½	16/7/29	8-8 c.c. <i>Chloral hydrate</i> .	" "	1 hour	?	"	S. 3599	Kept under observation. Still living.
"	24613	70	-/4/30	4-2 gm. intra-venously	Ether 2 c.c. by inhalation	1 hour	460 gm.	"	S. 3599	Kept under observation. Metaplasia in liver.
"	No number	74	-/4/30	4-2 gm. "	25 c.c. "	1 hour	368 gm.	"	S. 4167	Injected with blood from sheep, carrier of <i>Anaplasma ovis</i> and <i>A. capensis</i> . Died 18/6/30 of uraemia. Showed <i>A. ovis</i> . Injected with paratyphoid culture and died 6 days later.

TABLE I (continued).

Species.	Number	Weight, lb.	Date of operation.	Anaesthesia		Chloral H <sub>2</sub> O rate.	Weight of splcn.	Result.	Experiment	Subsequent History.
				Chloroform.	Duration.					
Ovine.....	24637	75	-/4/30	20 c.c. by intravenously	1 hour	4.5 gm. intravenously	380 gm.	Recovered	S. 4167	Injected with blood from sheep, carrier of <i>A. ovis</i> and <i>T. recondita</i> . Both parasites appeared after 24 and 60 days respectively. Injected with paratyphoid culture and died 20 days later.
"	23601	78	-/4/30	30 c.c.	1 hour	4.5 gm.	225 gm.	"	S. 4167	Injected with paratyphoid culture and died 2 days later.
"	28498	61	14/7/30	None...	2 hours	4 gm.	215 gm.	"	S. 3599	Kept under observation to study the formation of metaplasies in the liver. Died 29/10/32.
"	28578	56	14/7/30	None...	2 hours	4 gm.	119 gm.	"	S. 3599	Same as above No. 28498. Still living.
"	26331	71	-/4/30	None	3 hours	4 gm.	315 gm.	"	S. 4167	See above. <i>A. ovis</i> and <i>T. recondita</i> appeared. Discharged 9/8/31.
"	25305	69	17/7/30	None	3 hours	5 gm.	462 gm.	"	S. 4167	See above. <i>A. ovis</i> and <i>T. recondita</i> appeared.
"	32729	43	28/4/32	None.	4 hours	5 gm.	103.9 gm.	"	S. 3599 S. 4665	See above. Still living. Injected with blood from calf showing <i>A. centrale</i> but did not show parasites.
"	32732	38	28/4/32	None .....	4 hours	5 gm.	99 gm.	"	S. 4666	Injected with blood from calf showing <i>A. marginale</i> but did not show parasites.
"	33004	31	18/5/32	15 c.c. by inhalation	2 hours	2 gm.	45 gm.	"	S. 4664	Kept under observation. Died 31/8/32 of enteritis and pneumonia.
"	32674	42	18/5/32	None .....	2 1/2 hours	4 gm.	?	Died	—	Died under anaesthesia.
"	33013	27	26/5/32	8 c.c. by inhalation	2 1/2 hours	2 gm.	115 gm.	Recovered	S. 4664	Daily sputum examination. No parasites seen. Died 1/9/32 of gastritis.
"	33014	35	26/5/32	Ether 15 c.c. by inhalation	2 1/2 hours	3 gm.	68 gm.	"	S. 4665	Daily sputum examination. Discharged 1/2/33.
"	33044	35	26/5/32	"	2 1/2 hours	3 gm.	80 gm.	"	S. 4666	See ovine No. 32729. Discharged 1/2/33.
"	32704	40	11/5/32	None..	3 hours	5 gm.	214 gm.	"	S. 4665	See ovine No. 32732. Discharged 1/2/33.
"	32780	42	11/5/32	"	3 hours	5 gm.	209 gm.	"	S. 4664	See above.
"	28335	95	24/11/31	"	4 hour	6 gm.	460 gm.	"	S. 4596	Experiment in progress.
"	35000	87	6/2/34	"	5 1/2 hours	6 gm.	562 gm.	"	S. 3599	"
"	35004	62	6/2/34	"	6 hours	5 gm.	333 gm.	"	—	"
"	37862	54	6/2/34	"	3 hours	5 gm.	188 gm.	"	—	"
"	37429	53 1/2	6/2/34	"	3 hours	5 gm.	337 gm.	"	—	"

TABLE I (continued).

Species.	Number	Weight. lb.	Date of operation.	Anaesthesia.		Weight of spleen.	Result.	Experi- ment.	Subsequent History.
				<i>Chloral Hydrate.</i>	<i>Chloroform.</i>				
Caprine.....	8280	66	30/5/24	8 gm. per os.....	2 c.c. by inhalation	190 gm.	Recovered	S. 1602 S. 1613	Under observation for four months. Injected with blood from sheep harbouring anaplasma and showed a few anaplasma in blood smears. Died 31/10/32. Extensive degeneration of liver. Still alive.
" .....	8304	76	30/5/24	8 gm. "	2 c.c. "	?	"	S. 1602 S. 1613	Same as above. Still alive.
Canine.....	1127	46	13/9/32	<i>Eukodal.</i> 3 c.c. ....	<i>Pernoxeton.</i> 4-8 c.c.....	259 gm.	Recovered	S. 4790	Blood showed presence of microflaria 3 days after operation. Died 28/9/32 from intoxication due to bee stings.
" .....	1150	11½	7/10/32	1 c.c. ....	1-2 c.c.....	34 gm.	"	S. 4790	Daily smear examination. No parasites seen for 31 days. Then injected with blood from carrier of <i>P. canis</i> . After seven days parasites appeared and animal died 17/11/32 of bilious fever ( <i>P. canis</i> ).
" .....	1151	40	7/10/32	3 c.c.....	4-2 c.c. ....	250 gm.	"	S. 4790	Same as No. 1150 except that animal was treated on the 4th day after operation and recovered. Parasites appeared and died. Transferred to experiment S. 4931.
" .....	1073	43	13/9/32	3 c.c.....	3-8 c.c.....	125 gm.	"	S. 4931 S. 4790 S. 4931	Transmission of horse sickness. Still alive.
" .....	1051	13	19/1/33	Ether 15 c.c. ....	1-3 c.c.....	?	"	S. 4931	Same as No. 1151. Transmission of horse sickness. Still alive.
" .....	1044	41	19/1/33	Eukodal 3 c.c. ....	3-6 c.c.....	?	"	S. 4931	Died before injection with horse sickness, 31/1/33, result of perforating duodenal ulcer.
" .....	1143	12	19/1/33	Ether trace.....	1-2 c.c.....	?	"	S. 4931	Transmission of horse sickness. Still alive.

TABLE I (continued).

Species.	Number	Weight. lb.	Date of operation.	Anaesthesia.		Date of operation.	Weight of spleen.	Result	Experi- ment.	Subsequent History.
				<i>Chloral Hydrate.</i>	<i>Ether.</i>					
Blesbuck, <i>Damaiscus abyssinus</i>	32055	64	13/8/31	4 gm. intravenously	Few c.c. by inhalation	1½ hours	35½ gm.	Recovered	S. 4417	Injected with blood from bovine harbouring <i>anaplasma marginale</i> , and the animal died on the 11th day and could be demonstrated for 14 days. Other parasites did not appear. Splenectomised and showed relapse of anaplasmosis. Died as result of anaemia due chiefly to verminosis.
Blesbuck....	32054	60	4/12/31	5 gm. "	None . . .	2 hours	9	Died	S. 4417	Before splenectomy animal injected with blood from bovine harbouring <i>P. bigemina</i> , <i>P. matana</i> , and <i>P. marginale</i> , the animal died in the blood accompanied by slight anaemic changes but no clinical symptoms. Then splenectomised and died after operation.
Dulker, <i>Syl- vestris</i>	32806	36	7/4/32	3 gm. "	None. . .	1½ hours	80 gm.	Recovered	S. 4521	Died 8 days later, 5.32 as result of infection due to the contents of the spleen.
<i>L. grimmii</i> , Blesbuck. . .	33606	70	12/4/32	6 gm. "	45 c.c. by inhalation	2 hours	619 gm	"	S. 4636	Injected with blood from bovine harbouring <i>P. bigemina</i> , <i>P. matana</i> , and <i>P. marginale</i> . Spleen removed. Injected with blood from calf harbouring <i>A. marginale</i> which showed in the blood for 18 weeks. Injected with blue tongue virus. Showed no reaction but susceptible to blue tongue virus. Splenectomised. It showed typical blue tongue. Injected with blood from calf harbouring <i>aparocheeta theileri</i> . Parasites appeared on 6th day and were seen on 10th day.
Porcine.....	904	44	13/12/32	4-4 c.c. intravenously	<i>Ether and Chloroform.</i> 8 c.c. of each by in- halation	<i>Duration.</i> 3 hours	81 gm.	Recovered	S. 4914	Daily blood examination for 6 weeks. No parasites seen. Injected with horse sickness virus but showed no reaction. Still alive.
" .....	903	42½	13/12/32	4-4 c.c. "	None.....	3 hours	128 gm.	"	S. 4914	Same as No. 904.
" .....	905	39	15/12/32	4-4 c.c. "	Trace by inhalation	4 hours	120 gm.	"	S. 4914	Injected with blue tongue virus. No reaction still alive.
" .....	906	53	15/12/32	5-8 c.c. "	Trace by inhalation	4 hours	155 gm.	"	S. 4914	Same as No. 905.





## Section IX.

# Animal Husbandry.

- CURSON, H. H .. Studies in Native Animal Husbandry 12.  
A Wakonde Milk Pail.
- ROUX, L. L., AND VAN Cross-bred lambs under Eastern Transvaal  
RENSBURG, C. T. conditions.
- BOSMAN, V. ... .. The seasonal influence on Merino wool pro-  
duction.



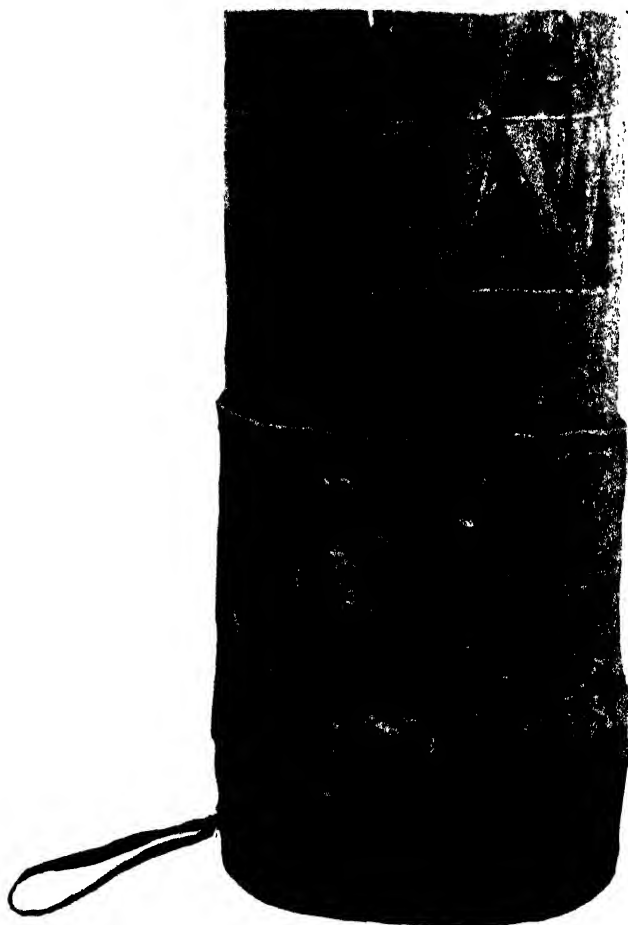
## **Studies in Native Animal Husbandry. (12) A Wankonde Milk Pail.**

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As mentioned in a former paper (Curson, Thomas and Neitz, 1930), the Wankonde or Wanyatyusa inhabit the country immediately north of Lake Nyasa. They are skilled in cattle management, and Fig. 1 shows the type of milk pail employed.



**Fig. 1.—Wankonde milk pail (height 23 cm., diameter 11 cm., and capacity 1,200 cc.)**

As will be observed, the vessel is quite unlike those shown in a previous study (Curson, Thomas and Neitz, 1933), being made of bamboo. Of particular interest are (a) the strengthening of the base with the skin taken from the limb of an ox, (b) the thin loop of hide by which the utensil is hung upside down when not in use, and (c) the common native pattern of triangles decorating the top half. The base of the pail is inserted into the skin "sock" or rather "mitten" usually taken from the forearm or lower thigh, when moist, so that when it dries it acts as a firm support.

The larger milk pail is made from a calabash (*ulukekwa*), both of which are kept far cleaner than by natives in the sub-continent, particularly the Damaras.

Other Central African tribes, e.g. Wakinga, also use the bamboo pail.

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## **Cross-bred Lambs under Eastern Transvaal Conditions.**

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### **INTRODUCTION.**

THE experiments reported here are part of a series of cross-breeding experiments which have been carried out by the Department of Agriculture at various institutions. The main objects in these experiments have been to determine the most suitable types of cross-bred lambs for various conditions and to investigate the requirements of management or treatment necessary for rearing fat-lambs.

The eastern districts of the Transvaal are suitable for Merino sheep farming. Schuurman *et alia* (1932) sounded a warning against the danger of the deterioration in the quality of the country's wool as a result of indiscriminate cross-breeding, but well organized cross-breeding enterprises were advocated where conditions permit, such as in high rainfall areas in which were included the maize growing districts of the eastern Transvaal.

In the eastern Transvaal, the most influential factors in any type of sheep farming are feed and parasites. Merino farmers in these districts regulate most of their lambing to take place in the autumn, April and May. This practice is favoured largely because of the set back spring-born lambs experience due to internal parasites. If the same practice is applied to cross-breeding, the lambs have to be finished off during winter months when feed is scarce. On the other hand, cross-bred lambs born during the spring, September and October, have an abundance of natural pasture available during the stage of growth when the highest rates of gain are made, so that, when such lambs have attained a desirable weight at four or five months of age, they should receive special attention from all markets demanding genuine lamb during January, February and March. Lochner and Beyleveld (1932), in discussing the English mutton and lamb markets, point out that various grades of lamb demand two to three pence per pound more than do the corresponding grades of mutton. They also state that the best time for marketing lamb in England is during the English winter months, prices for genuine lamb being at the peak during March and April.

At present, in this country, the relative scarcity of the mutton breeds restricts the choice of breed of ram for cross-breeding purposes. However, it appears logical that, as the demand for particular breeds of rams develops, it will become economically possible for breeders in various parts of the country to cater to the particular demands of their districts.

Results have been recorded upon the use of Suffolk rams for cross-breeding purposes, but most of these experiments involve the use of Blackhead Persian and unimproved types of ewes. However, Maré (1934) reported results with Suffolk and Rowney Marsh rams mated to Merino ewes under Karroo conditions. Rose (1932) in considering a general policy, is of the opinion that while practically all the British breeds are suitable for mating to Merino ewes for mutton crosses, it would be well to confine choice to the white-faced, white-woolled breeds in order to maintain colour purity of fleece. Bartel and Johnstone (1934) studied the rearing of fat-lambs under Western Province conditions for which purpose they mated Merino ewes to the following breeds of rams: Dorset Horn, Texel, Border Leicester, German Merino, and Corriedale. Colebatch and Scott (1928) have studied in great detail the suitability of many types of lambs under South Australian conditions and their detailed comparisons are considered excellent guides. They concluded that half-bred long-wool-Merino ewes are best as mother stock and that, considering both lambs and wool, the most profitable returns can be obtained from Southdown  $\times$  Border Leicester-Merino and Dorset Horn  $\times$  Border Leicester-Merino crosses. When Merino ewes were the mother stock, the investigators found that the use of Border Leicester, English Leicester, and Lincoln rams proved most remunerative, and that the use of these rams on Merino ewes was infinitely more remunerative than the use of the short and medium woolled British breeds of rams on Merino ewes.

#### OBJECTS OF THE EXPERIMENT.

In view of the success of Border Leicester and Southdown rams for cross-breeding purposes in other countries, it was decided to include these breeds in the Department's scheme of cross-breeding experiments. While the use of Ryeland rams has not received much attention in the past, it is a breed of good mutton qualities; also, the breed is devoid of colour. It is very probable that this latter breed when bred to Merino ewes or to long-wool-Merino half-breds, will give satisfactory results.

The following are the main features of the experiments conducted at the Veterinary Research Station, Ermelo:—

1. A comparison of the following types of cross-bred lambs:—
  - (a) Border Leicester-Merino.
  - (b) Ryeland-Merino.
  - (c) Southdown-Merino.
  - (d) Merino (flock).

2. The possibility of rearing suitable fat-lambs from spring-born lambs when no supplementary feeding is practised.

### PLAN OF THE EXPERIMENT.

Two rams of each of the British breeds, Border Leicester, Ryeland, and Southdown, were imported from England, and they were at this Experiment Station for three months before mating commenced. The Merino ram used was obtained from the Grootfontein School of Agriculture, Middelburg, Cape. All rams were about two years old when mated.

The intention was to mate fifty Merino ewes to each ram; therefore, a total of 350 ewes, varying in age from two years to aged, was drafted into the experiment. Due to the shortage of ewes, many of those used were too old for the purpose. The unsuitability of old ewes is discussed in this report.

In order to ensure successful lamb crops, the system of "hand" or controlled serving was practised. It will be seen from the results that a great deal of information was obtained by adopting this system of mating. The ewes were brought to the yards at 6 a.m. daily during the mating season of six weeks, for daily testing for oestrus which was done by means of vasectomised teasers. Ewes showing oestrus were kept for service during the day. The selection of ewes for the various rams was at random, no attention being paid to size and age. Each ewe was given one service. All ewes were tested daily for a recurrence of oestrus throughout the mating season and for a further twenty days subsequent to the termination of the mating season. During the mating season, ewes showing a recurrence of oestrus were, whenever possible, mated to their original rams.

The rams were kept in good vigorous condition; their monthly weights served as a guide of their condition.

The ewes were given what was considered good farm management. They were put on green oat grazing during the winter months and an attempt was made to improve the grazing as lambing time approached. Unfortunately, due to adverse weather conditions, the available green grazing was not sufficient to maintain the ewes in high condition, with the result that the old ewes showed considerable decline in condition and they proved detrimental in the interests of the experiment.

The ewes were dosed regularly at twenty-one day intervals with Government Wireworm Remedy until six weeks before lambing was to commence.

A lick consisting of two parts of bone meal and one part of salt was supplied to the flock at the rate of about five ounces per sheep per week.

The flock was well attended during the lambing season. Newly born lambs were identified by the ear tags and special body markings on the ewes; the latter marks were given at the time of mating. All lambs born were ear-tagged and weighed; this work was done daily.

Due to the shortage of green oat grazing, the ewes were lambed down on a "burn" which improved as lambing progressed. At the termination of lambing the grazing was good.



The lambs were castrated and docked in several batches when they were two to three weeks old.

The entire flock of ewes and lambs was run together. The lambs had access to the lick supplied to the ewes; the amount issued was increased when deemed necessary.

The dosing of the flock was resumed as soon as possible after the lambing season, when ewes and lambs were dosed with Government Wireworm Remedy at twenty-one day intervals.

The lambs were weighed at twenty-one day intervals; the weighing periods corresponded with dosing periods. At the commencement, the young lambs were starved for four hours before being weighed; the starvation period was subsequently extended to twelve hours.

Due to the danger of loss through Blue Tongue, all ewes and lambs were inoculated when the lambs were approximately ten weeks old. It proved to be an exceptionally bad blue tongue season; however, no lambs were lost through this disease.

All sheep on the Experiment Station were dipped for keds in an arsenical dip; the ewes and lambs were dipped when the latter were approximately three months old.

## RESULTS.

Table I contains the results of controlled serving.

It is seen from Table I that abnormally large percentages of ewes were not impregnated at the first service.

These percentages were as follows:—

Ewes mated to Border Leicester rams ... ..	32·1 %
Ewes mated to Ryeland rams ... ..	37·9 %
Ewes mated to Southdown rams ... ..	70·6 %
Ewes mated to Merino ram ... ..	12·0 %

In the case of the Southdown rams the recurrences of oestrus after service are seen to have been very exceptional. In order to prevent overtaxing the Southdown rams, many of their ewes were not given a second service upon the recurrence of oestrus. However, if a second service had been given to all such ewes, the above percentage of 70·6 would have been larger. The two Southdown rams gave a total of 184 services and, as is seen in Table I, only three ewes were impregnated.

It is of interest to note that all the rams showed a marked keenness to mate and that the Southdown rams were particularly active in this respect. Ryeland ram No. 36448 developed an abscess on the ear and became reluctant to serve. The ram was withdrawn for a period, consequently the number of ewes served by this ram is small.

TABLE I.  
*Serving Data.*

Breed of ram.	Ram No.	First service.	Second service.	Third service.	Recur- rences not served.	Ewes sup- posedly settled.	Number of lambs born.	Number of ewes lost between mating, lambing.	Number of ewes supposedly settled that did not lamb.	Percentage of ewes supposedly settled that did not lamb.
Border Leicester.....	36445	58	17	2	1	57	39	2	16	28 07
Border Leicester.....	36446	51	18	1	2	49	31	2	16	32 65
Ryeland.....	36447	67	29	1	9	58	46	2	10	17 24
Ryeland.....	36448	28	7	0	11	17	10	2	5	29 41
Southdown.....	36449	45	32	11	36	9	2	0	—	—
Southdown.....	36450	47	33	16	41	6	1	0	—	—
Merino.....	35760	50	6	1	3	47	31	3	14	29 78

In work on the fundamentals of fertility in Merino sheep Quinlan, Maré, and Roux (1932) found that when ewes were served at various periods from the onset of oestrus up to thirty hours after the onset of oestrus, the fertility established ranged between 70 and 100 per cent. The authors also indicated what differences in fertilizing power might exist in mating clinically normal rams to clinically normal ewes. Even when the controlled service method of mating was employed with thirteen rams, it was found that the largest number of ewes fertilized at the first service was 84.6 per cent. In view of these findings, it must be considered that in the case of the matings with the Merino ram in this experiment, the 12 per cent. of unfertilized cases after the first service is not exceptional.

When it became obvious that an abnormally large number of ewes was returning for service, all the rams used in the experiment were sperm tested. However, when these tests were made, the Border Leicester, Ryeland, and Merino rams showed abundant and active normal spermatozoa in the microscopic examination of their semen. The breeding results confirmed the finding of the sperm tests, in that the percentages of pregnancies subsequently established by the above three breeds of rams were satisfactory. Apparently the Border Leicester and Ryeland rams were in a condition of temporary infertility at the commencement of the mating period and these rams developed a higher degree of fertility after a few weeks of serving. A similar experience of infertility with a Border Leicester ram was reported by Bartel and Johnston (1934), although in this case sperm tests were not carried out and the subsequent state of fertility of the ram was not reported.

The sperm tests of the two Southdown rams revealed that they were sterile due to scarcity of spermatozoa and there was also a disintegrated condition of the majority of the spermatozoa present. Throughout and subsequent to the mating period, these two rams were sperm tested and these tests revealed that no change in their condition of sterility had taken place.

A maximum lambing percentage is an important factor in an enterprise such as fat-lamb production, as high fertility in rams contributes a great deal towards the net return per hundred ewes. The unknown presence of a sterile ram reduces the proportion of rams to ewes; however, as ewes may show oestrus and may be served twice or thrice during the usual mating period of six weeks, the lambing results, considered from the point of view of the rams only, will depend upon the percentage of rams used and the activity and fertility of the remaining rams. Rams, and especially young rams, which have been imported from foreign countries or even from districts of marked physiographical differences may require acclimatization, or they may acquire normal fertility only after their sexual energies have been drawn upon for several days. If this irregularity is suspected, it would appear to be best to commence mating seven to fourteen days earlier than is the usual custom and to extend the mating period to eight weeks. Should there be a material difference between the earlier and the later born lambs, due to a low percentage

of fertility during the earlier part of mating or because of the prolonged period of mating, lambs could be disposed of in two or three even batches, as there is a considerable remunerative advantage in marketing pens or groups, the lambs of which are even in size and condition.

The fertility of rams can be assured only by trial breeding or by sperm tests.

The number of ewes which showed a recurrence of oestrus when mating was discontinued, is given in Table I. In spite of the fact that a number of ewes did not show oestrus, they eventually proved to be non-pregnant; this occurred in 24.11 per cent. of the ewes mated. The cause of such abnormal inactivity is not definitely known, but it may have been influenced greatly by the large number of aged ewes used in the experiment. It has been observed that Merino sheep at this Station show normal sexual activity during March, April, and May.

The lambing data are given in Table II.

TABLE II.  
*Lambing Results.*

Breed of ram.	Number of ewes mated.	Lambs born.		Deaths: birth to weaning.		Lambs weaned.	
		Number.	Percentage.	Lambs.	Ewes.	Number.	Percentage.
Border Leicester.	109	76	69.90	7	4	69	63.30
Ryeland....	95	56	58.94	3	3	53	55.79
Southdown.....	92	3	3.27	0	0	3	3.27
Merino.....	50	31	62.00	3	4	28	56.00

The low lambing percentages reflected in the above table have been explained in the preceeding paragraphs.

The deaths of lambs were due largely to the inability of old ewes to mother the lambs, while the majority of losses among the mature sheep were old broken-mouthed ewes.

Because of the small number of Southdown cross-bred lambs, these lambs are being disregarded in subsequent discussions.

It is of interest to demonstrate the effect of weight of sire and dam upon the progeny; Table III has been constructed for this purpose.

TABLE III.

*Weight of Sire and Dam in Relation to Weight of Lamb.*

Breed of ram.	Weight of ram.	Range of ewe weights.	Average weight of ewes.	Number of ewes mated.	Number of lambs born.	Average birth weight of lambs.	Average weight of lambs at 3½ months.
	lb.	lb.	lb.			lb.	lb.
Border Leicester.	168	66-75	72 0	5	5	8 0	40 4
Border Leicester.	206		69·2	6	6	8·7	46·1
Ryeland.....	124		69 2	11	11	7·7	40·7
Ryeland.....	127		—	—	—	—	—
Merino.....	118		69 9	10	10	8 1	42 8
Border Leicester.	168	76-85	72·0	11	11	7 8	44 8
Border Leicester.	206		79 8	9	9	9·1	49 2
Ryeland.....	124		80 2	12	12	8 4	47 2
Ryeland.....	127		81·8	3	3	8 3	40 6
Merino.....	118		79 9	6	6	7 8	46·1
Border Leicester.	168	86-100	92 6	10	10	8·7	51·0
Border Leicester.	206		94 9	11	11	9 6	56 8
Ryeland.....	124		93 8.	12	12	8 9	47 1
Ryeland.....	127		91 7	3	3	9 5	50·6
Merino.....	118		93 4	5	5	7 9	47·9

The weights of the rams and ewes were taken at the time of mating when they were in good condition. The analysis indicates that in the case of the British breeds of rams, heavy rams tend to produce heavy lambs and that the ewes exert a similar influence. In addition, it is revealed that the heavy lambs at birth retain the advantage of weight at three-and-a-half months of age. However, in the Merino breed these relationships were not as striking. There appears to be every justification to maintain that large roomy Merino ewes are most suitable for cross-breeding and that small stunted Merino ewes are undesirable.

The average weights of the three types of lambs are given in Table IV at three weekly periods and up to the age of twenty weeks, while the average daily gains are presented in Table V.

TABLE IV.  
*Average Weights of Lambs.*

Type of lamb.	Birth weight.	6 weeks, 15/11/33.	9 weeks, 6/12/33.	12 weeks, 28/12/33.	15 weeks, 18/1/34.	18 weeks, 8/2/34.	20 weeks, 17/2/34.
	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Border Leicester-Merino.....	8 5 (70)	32 3 (70)	38 0 (69)	44 4 (68)	51 6 (69)	51.1 (68)	51.3 (67)
Ryeland Merino.	8 6 (56)	30 1 (56)	33 2 (56)	42 0 (55)	45 6 (54)	44 6 (53)	45.2 (46)
Merino (flock)...	7.8 (31)	25 0 (31)	32 1 (30)	40 0 (30)	44 3 (30)	41 6 (29)	38.0 (26)

NOTE.—In the above table the number of lambs involved in the averages is given in brackets.

TABLE V.  
*Average Daily Gain.*

Type of lamb.	Birth to 6 weeks.	6-9 weeks.	9-12 weeks.	12-15 weeks.	15-18 weeks.	18-20 weeks.	Birth to 20 weeks.
	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Border Leicester-Merino.....	0 57	0 27	0 30	0 34	-0 02	0 01	0 28
Ryeland-Merino.	0 51	0 14	0 41	0 17	-0 04	0 04	0.24
Merino (flock)...	0 41	0 34	0 38	0 20	-0 12	-0 25	0 20

It is seen from the above two tables that the growth of the lambs of all types was erratic and unsatisfactory. It was explained previously that inoculation and dipping were necessary. The lambs were inoculated on 6th December, 1933, and they were dipped on 12th January, 1934, and 2nd February, 1934. It is not considered that these operations had a serious adverse effect upon the growth of the lambs. However, a factor which must have inhibited growth seriously was worm infestation. The lambs were found to be heavily infested with tape worms during the latter part of December and, as the season was a wet one, considerable difficulty was experienced in keeping the lambs free of worms. In spite of an abundance of pasture during January and February, the lambs made no progress.

Measures for overcoming adverse influential factors and for stimulating the growth rate will be made under the heading "Summary and Conclusions".

The data presented in Table IV and V clearly indicate that the Border Leicester-Merino lambs made the most satisfactory gains and that the Ryeland-Merino lambs made considerably better gains in weight than did the Merino flock lambs.

The results of transporting, slaughtering, and marketing the wether lambs are reflected in Table VI.

## CROSS-BRED LAMBS UNDER EASTERN TRANSVAAL CONDITIONS.

TABLE VI.

*Market Results.*

Type of lamb.	Per cent. lamb. fit.*	Number dispatched.	Weight before dispatch.	Weight at abattoir.	Loss in transit.	Carcass weight.	Dressing per cent.	Carcass grade frequencies.			Sale price.
								Good.	Medium.	Common.	
Border Leicester- Merino.....	96	36	lb. 57 6	lb. 50 8	per cent. 11 9	lb. 24 1	47 5	4	17	15	s. d. 12 0
Ryeland-Merino....	86	19	50 4	47 9	5 0	22 4	46 7	0	7	12	11 2
Merino (flock).....	83	10	49 0	45 1	7 9	20 8	46 1	0	1	9	10 6

\* NOTE.—1. Only wether lambs were sent for the slaughter tests; the ewe lambs were retained for further work.  
2. The abattoir weights were taken 48 hours after dispatch.

While the majority of the lambs had not attained a desirable market weight at twenty weeks, it was the object to obtain marketing and slaughtering results of such lambs off pasture and without supplementing feeding. All lambs above a minimum weight of 40 pounds were despatched.

By consulting Table VI, it is seen that the Border Leicester-Merino lambs showed a decided advantage with respect to the percentage of lambs fit for despatch, and while their percentage loss in transit was highest, this type was superior in carcass grades. In this latter respect, the Merino flock lambs were particularly poor.

The dressing percentages of both cross-bred types were poor for lambs of their breeding, but this undoubtedly was due to lack of "condition".

The lamb carcasses were sold on the open market and prices from 5½d. to 6½d. per pound were realised. Due to the method of selling, the prices indicated in the above table are not good indications of condition and quality.

Blemishes which were sufficient to disqualify carcasses for export purposes were found on many of the carcasses. The awns of *Trachypogon polymorphus* had penetrated the skin and become lodged in the subcutaneous tissues, resulting in large bruise-like patches and in many instances abscesses.

The carcasses were graded after they were allowed to "set", after which each carcass was examined with respect to conformation, finish, and shape of hindquarters, a definite scale of points being used for the classification. The results of such examinations are reflected in Table VII.

TABLE VII.  
*Examination of Carcasses.*  
*Frequencies of Scale of Points.*

Type of lamb.	Confirmation.							Finish.							Shape of hindquarters.						
	2	3	4	5	6	7	8	3	4	5	6	7	8	3	4	5	6	7	8		
Border Leicester-Merino.	0	3	6	8	11	6	2	5	5	7	12	4	3	2	9	8	13	2	2		
Ryeland-Merino.....	1	2	6	5	2	3	0	4	7	3	3	2	0	3	4	8	1	3	0		
Merino (flock).....	0	5	3	1	1	0	0	5	1	3	0	1	0	4	5	0	1	0	0		

The mean values of the three features for which the carcasses were examined are given in Table VIII.



TABLE VIII.  
*Mean Values of Carcasses.*

Type of lamb.	Conformation.	Finish.	Shape of hindquarters.
Border Leicester-Merino.....	5.47	5.358	5.276
Ryeland-Merino.....	4.74	4.579	4.842
Merino (flock).....	3.80	4.100	3.800

The significance of the differences of the above mean values have been obtained by further statistical analysis, and the results may be summarised as follows:—

1. *Conformation.*

- (a) Differences between B. Leicester crosses and Ryeland crosses were not quite significant.
- (b) Differences between B. Leicester crosses and Merinos were highly significant.
- (c) Differences between Ryeland crosses and Merinos were significant.

2. *Finish.*

- (a) Differences between B. Leicester crosses and Ryeland crosses were significant.
- (b) Differences between B. Leicester crosses and Merinos were highly significant.
- (c) Differences between Ryeland crosses and Merinos were insignificant.

3. *Shape of Hindquarters.*

- (a) Differences between B. Leicester crosses and Ryeland crosses were insignificant.
- (b) Differences between B. Leicester crosses and Merinos were highly significant.
- (c) Differences between Ryeland crosses and Merinos were significant.

In the above summary, the term “significant” means that the probability that the difference could be due to chance (random sampling) is less than five per cent., while “highly significant” indicates that the probability is less than one per cent.

SUMMARY AND CONCLUSIONS.

1. Controlled serving revealed irregularities with regard to fertility in rams and ewes.

The fertility of a ram can be assured only by breeding trials or by sperm tests.

Old Merino ewes are unsatisfactory and uneconomical for cross-breeding purposes. Large framed, roomy ewes give best results.

While controlled or "hand" serving is advocated in order to obtain maximum results, it is considered that free mating in small camps may be practised with advantage when facilities permit. The latter system of mating has been employed during the 1934 mating season in the continuation of these experiments.

2. The lambs of all types did not attain a desirable weight at twenty weeks of age.

The Border Leicester-Merino wether lambs weighed 57.6 lb. at twenty weeks of age and 96 per cent. were fit for despatch; they dressed 47.5 per cent. and 11.1 per cent. of the carcasses were graded as "good", 47.2 per cent. "medium" and 41.7 per cent. "common".

The Ryeland-Merino wether lambs weighed 50.4 lb. at twenty weeks of age and 86 per cent. were fit for despatch; they dressed 46.7 per cent. and none of the carcasses were graded as "good", 36.8 per cent. were "medium", and 63.2 per cent. "common".

The Merino flock wether lambs weighed 38.0 lb. at twenty weeks of age and 83 per cent. were fit for despatch; they dressed 46.1 per cent. and none of the carcasses were graded as "good", while 10 per cent. were "medium" and 90 per cent. "common".

There was no significant difference between the conformation and the shape of hind quarters of the Border Leicester and the Ryeland crosses, but the former crosses were significantly better in finish.

The Border Leicester crosses were infinitely superior to the Merino flock lambs with respect to conformation, shape of hindquarters, and finish.

There was no significant difference between the finish of the Ryeland crosses and the Merino flock lambs, although the former were superior in conformation and shape of hind quarters.

3. Drastic control measures against internal parasites, especially wireworms and tapeworms, are essential if September lambing is to be a success under eastern Transvaal conditions.

The use of copper sulphate and tobacco extract as advocated by Mönnig (1932) is recommended. This treatment will be given a thorough test during the next season.

4. Due to the poor finish of lambs reared on grass, it appears that a supplementary feeding period will be warranted. Results indicate that supplementary feeding may be commenced when the lambs are twelve weeks old. Systems of supplementary feeding will be conducted with the 1934 lamb crop.

5. The experiment reported must be considered as the preliminary work of experiments which will be extended over a number of years.

The experiments during the next year will include the use of Corridale rams.

When the half-bred ewes, which have been retained, are fit for breeding, they will be included in the further work at this Station.

The authors wish to acknowledge the assistance of Dr. J. G. Bekker and Mr. G. N. Murray, Research Officers, Onderstepoort, who undertook the grading and measuring of the carcasses at the Pretoria Abattoir. Appreciation is also expressed to Mr. A. P. Malan, Statistician, Onderstepoort, for the analysis of the slaughtering results.

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## **The Seasonal Influence on Merino Wool Production.**

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THE fleece of the Merino sheep, unlike that of many other domestic mammals in which winter and summer changes take place, grows continuously from month to month and from year to year.

In the horse, cow, and donkey, seasonal variations occur (Duerden and Whitnall, 1931). Among the ovidae, seasonal shedding takes place in *Ovis ammon poli* (Crew, 1921), the Blackhead Persian (Boyd, 1927), the Auodad and Mouflon sheep (Duerden and Seale, 1927), the Welsh Mountain sheep (Roberts, 1926), and the British mountain breeds (Duerden, 1927, 1929), all these being representative of the more primitive type of coat.

The Merino sheep, however, with its specialized fleece, has lost the power of seasonal shedding and, unlike its ancestors, has continuously growing follicles. Even though no shedding takes place, it is of interest to know whether its fleece is influenced by seasonal changes, and an analysis is outlined of the winter and summer growths of three Merino wethers that were maintained at the Grootfontein School of Agriculture, Middelburg, Cape, where extreme winter and summer climatic variations occur. Since a change in nutrition has a marked influence on Merino wool growth (Maré and Bosman, 1934), the experimental sheep were stall-fed.

### **MATERIAL AND METHODS.**

The material for study was obtained from three forty-months-old Merino wethers which from birth were stall-fed on a balanced ration and were never shorn. The changes in winter and summer pasturage were thus eliminated, and it was possible to study the same staple of wool for consecutive seasons for three years, and also to compare the yearly growth for the first three years of the sheep's life.

The staples from the sheep were approximately twenty-five centimeters or ten inches in staple length. The first seven months' growth of the staple was not taken into account, since this portion, representing the coat of the lamb, is not comparable with the fleece grown by the adult merino (Botha, 1930). The remainder of each staple was divided into cuttings corresponding to the winter and summer growths. The guide for identifying the regions was obtained from

# SEASONAL INFLUENCE ON MERINO WOOL PRODUCTION.

clippings taken at monthly intervals and used subsequently in a study of the monthly rate of growth. It was thus possible to identify accurately the seasonal and yearly growths in the same staple and to determine any changes in the regions.

The cuttings of the winter and summer growths that were in the greasy state were scoured by the benzene-saponin method (Miller and Bryant, 1932), and dried to constant weight in regain bottles (Barrit and King, 1926). The dry weights are summarized in Table 1. An estimate of the number of fibres in each cutting was made and the fibre fineness calculated. The method is similar to that used by Roberts (1927) for estimating fibre fineness, and takes into account the mean cross-sectional area of the fibres.

## THE SEASONAL CHANGES IN THE KARROO.

In the Karroo there are varying factors that influence climate, such as temperature, humidity, sunshine, wind, etc. Meteorological observations for temperature, rainfall, sunshine, and wind at the Grootfontein School of Agriculture were available, and each of these shows seasonal variations as summarized in Table 1.

Table 1.—*The Wool Analyses of Winter and Summer Growth of Three Merino Sheep that were Never Shorn and the Seasonal Meteorological Records at the Grootfontein School of Agriculture, Middelburg, Cape.*

	Winter, 1931.	Summer, 1931/32.	Winter, 1932.	Summer, 1932/33.	Winter, 1933.	Summer, 1933/34.
<i>Sheep No. 1.</i>						
Dry weight (gms.).....	·4260	·4288	·4291	·4253	·4285	·4265
Fibre fineness ( $\mu$ ).....	20·8	20·9	20·9	20·7	20·9	20·8
<i>Sheep No. 2.</i>						
Dry weight (gms.).....	·3978	·3959	·3988	·3975	·3984	·3960
Fibre fineness ( $\mu$ ).....	18·7	18·6	18·7	18·7	18·7	18·6
<i>Sheep No. 3.</i>						
Dry weight (gms.).....	·4455	·4480	·4472	·4450	·4463	·4485
Fibre fineness ( $\mu$ ).....	18·1	18·2	18·1	18·1	18·1	18·2
<i>Meteorological Records at Grootfontein (Averages).</i>						
Maximum (degrees F.)....	62·7	83·7	64·7	84·9	63·0	77·9
Minimum (degrees F.)....	33·5	53·1	36·5	51·6	31·3	53·3
Sunshine, hours.....	8·0	11·2	8·5	11·1	8·2	9·2
Rainfall (inches).....	3·21	6·60	1·08	3·32	·82	12·5
Wind (miles per hour)....	3·5	3·6	4·2	3·5	5·0	2·9

As regards temperature, an extreme variation, typical of the Karroo, is shown. The four winter months of May, June, July, and August were regarded as the coldest period against the four summer months of November, December, January, and February, which constitute the hottest period of the year. Although the average maximum

and minimum temperatures in the table vary from 31·1° F. to 84·9° F., larger individual variations were recorded and the maximum often reached 90° F. in the shade and the minimum temperature as low as 15° F. or 17 degrees of frost.

As regards sunshine, the meteorological observations show a seasonal variation of from 8·0 to 11·2 in sunshine hours. This figure, however, does not indicate the intensity of the sunlight, but records concerning the latter were not available.

Observations on rainfall for the same four-monthly periods show a variation of from 1·08 inches to 12·5 inches. Rainfall influences the nutritional value of the pasturage, but in this case the sheep were stall-fed, and the rainfall therefore does not have the same influence on wool growth as when the animals are run on the veld.

As regards wind force, the winter months of 1932 and 1933 show a higher rate per hour than the summer months of these years. Although the average of the wind force is not higher than five miles per hour, twenty miles per hour was frequently recorded.

The variations in the seasonal factors are shown to be appreciable, and whether these factors have an influence on Merino wool production has been a controversial topic among wool farmers.

#### EXPERIMENTAL RESULTS.

The wool analyses of the experiment are summarized in Table 1. As regards the dry weights of the wool cuttings, there is no significant difference between the consecutive seasons or between the consecutive years, the difference shown being well within experimental error. As regards fibre fineness no difference is shown between the winter and summer growths, nor between the yearly growths. It must therefore be concluded that *the seasons have no influence on wool fineness in the Karroo, provided the feed is kept constant*. There is also no difference in fibre fineness from year to year for the first three years under similar conditions of feed. (The lamb's coat representing the first seven months of growth being disregarded.)

#### CONCLUSION.

The Merino sheep does not show seasonal changes in wool fineness in a varying Karroo climate provided the feed is kept constant.

As regards length, several workers have shown that the rate of growth is constant from month to month (Burns, 1931; Fraser, 1931) and from year to year (Duerden and Maré, 1931) if the feed is kept constant. It follows, therefore, that in the Karroo at least there is no seasonal influence on the dimensional attributes of fineness and length which affect the volume of wool produced by Merino sheep, provided the nutrition is not changed.

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## Section X.

# Poultry.

VAN MANEN, E., AND   The enzymic activity of egg-white. Its  
RIMINGTON, G.         bearing on the problem of watery whites.





## **The Enzymic Activity of Egg-White. Its Bearing on the Problem of Watery-Whites**

By

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ONE of the hypotheses advanced as an explanation of "watery-whiteness" in eggs is that there is present in the albumen of such eggs enzymes of proteolytic nature, by the action of which the protein becomes disintegrated, thereby decreasing in viscosity.

Experiments designed to test this hypothesis were commenced as far back as the spring of 1933, but the results then obtained were considered insufficient to substantiate the view, and the experiments were discontinued.

A publication appeared recently however by A. K. Balls and T. L. Swenson of the Bureau of Chemistry and Soils, Washington D.C. (1934), in which the claim was made that a proteolytic enzyme is present in egg-white, particularly in thick albumen, and that "the disappearance of thick white from eggs in storage is due to a slow proteolysis catalysed by tryptic proteinase".

In view of the fact that the methods employed by these authors appeared in our opinion to be open to criticism, it was decided to investigate the problem using a suitable technique.

In the first place, a repetition of the Willstatter titration method employed by Balls and Swenson convinced us that the accuracy is insufficient for the detection of such small changes as were to be expected. In no instance could increases of the order observed by the American workers be recorded; the end-points were never particularly sharp in such large volumes. Attempts were made to increase the accuracy by titrating to a definite pH standard in a comparator instead of "to a faint but true blue which required a little practice to recognise" but without much success.

The choice of the above method for the purpose of the investigation would seem moreover to have been singularly unfortunate. Recognising, as it does, any increase in carboxyl groups, it would fail to distinguish between lipolytic and proteolytic activity. The presence of an active lipase in both egg yolk and egg white has been demonstrated by Koga (1923) among others.

## ENZYMIC ACTIVITY OF EGG-WHITE.

Any attempt to detect the presence of small quantities of proteolytic enzymes (proteases, ereptases, etc.), in egg albumen demands the employment of a sensitive micro-method which is specific for the determination of peptide cleavage. Of the various methods available, the well-known formol titration technique appeared to us to be the most suitable. Using decinormal carbonate-free sodium hydroxide, contained in an automatic micro-burette graduated in twentieths of a cubic centimeter, and titrating to a standard pH value (pH 8.4) with the help of a Cole-Onslow comparator, no difficulty was experienced in attaining an accuracy of  $\pm 0.02$  c.c. This is clearly evident from the protocols recorded.

### EXPERIMENTAL.

Having in our previous experiments (not recorded) employed only the one substrate gelatin, which is frequently used in the determination of tryptic activity, and obtained negative results, it was decided to extend the scope of the investigation by including such substrates as casein and peptone and by working over a wide pH range, any erepsin-like ferment would be expected to attack casein and peptone but not gelatin. No search was made for lipolytic or other types of enzyme.

#### *Albumen solutions.*

Various numbers of eggs were used as recorded, either new laid (1 hour old), fresh or old from white Leghorn pullets, intensively housed and all fed on a grain and mash ration. The albumen was separated into the two portions, thick and thin by the sieve method of Holst and Almquist (1931). 3 c.c. portions of either fraction were diluted to 10 c.c. with water and allowed to stand for 30 minutes at 35°.

In the majority of these experiments, the precipitated mucin was centrifuged off after shaking the diluted solution with glass beads, thereby avoiding the difficulty of removing a representative sample of the digestion mixture by the pipette, and excluding the possibility of precipitated particles adsorbing the indicator.

It is noteworthy that the proportion of thick to thin albumen did not appear to be markedly different in normal and so-called watery-white eggs, except those which were very stale, leading us to suspect that physical variations in organisation of the protein phase may be intimately bound up with the phenomenon of watery-whiteness.

#### *Substrates.*

Two and 5 per cent. casein solutions were prepared in the usual way by grinding the protein with the requisite amount of alkali, and diluting to volume. The pH was adjusted if necessary to 8.4.

Two per cent. gelatin was prepared by dissolving commercial gelatin in warm water (60°) and adjusting the pH.

2 per cent. peptone was similarly prepared from Parke, Davis & Co., "Bacterologic Peptone".

A few drops of toluol were added and each stoppered solution kept in the ice box when not in use

*Buffers.*

A range of phosphate and of borate buffers were prepared from Clark and Lubs tables; the solutions were not, however, diluted as a high buffering power was desired. In the comparator a borate buffer of pH 8.4 was employed.

*Trypsin.*—An active trypsin solution was prepared from pigs pancreas according to Cole (1926). Its activity was tested upon casein (see control experiment No. 4).

A glycerol extract of pancreas was also prepared and this trypsinogen utilised to check the activity of the enterokinase preparation (calcified milk method).

Enterokinase was prepared following Balls and Swenson (1934).

An Erepsin solution was prepared by grinding pig's intestinal mucosa with sand and water, straining and filtering. Its activity is demonstrated in control experiments Nos. 6 and 7.

*Formol Solution.*—This was made up according to Cole (1926). One drop of 1 per cent. alcoholic phenolphthalein solution being added per c.c. and sufficient sodium hydroxide solution to bring to pH 8.4. The reaction was carefully adjusted each day.

*Titration Technique.*—The digestion mixtures were invariably made up by mixing, in small stoppered flasks, 10 c.c. of the diluted egg albumen, 10 c.c. of substrate solution and 15 c.c. of buffer and adding 4 drops of toluol, an amount previously determined as sufficient to inhibit bacterial activity.

In cases where kinase was used, 1 c.c. of this solution replaced 1 c.c. of buffer. The buffering power was sufficiently strong to enable this to be done without affecting the final pH of the mixtures. The solutions were left in a constant temperature water bath at  $\pm 37^{\circ}$ .

An aliquot of 10 c.c. was withdrawn for titration at zero time. To this was added 10 drops of 1 per cent. alcoholic phenolphthalein solution and decinormal sodium hydroxide run in until the colour as seen in the comparator matched the standard of pH 8.4. Five c.c. of formol solution was now added and the mixture again titrated to pH 8.4, the quantity required being a direct measure of the amount of amino nitrogen present. The delicacy of the titration was such that a difference of one drop, equal to 0.02 c.c., could be easily distinguished.

After a time interval which varied in different experiments from 1 to 24 hours, a second sample was removed and similarly titrated, a final sample being taken at the close of the experiment. The titration differences between the zero and first and second time intervals are recorded in the protocols.

## RESULTS.

*Control Experiments.*

The control experiments of Table I served to demonstrate the reliability of the method as far as reproductivity of results was concerned and the absence of bacterial interference. Even in the

presence of enterokinase no proteolysis occurred in the various substrates after 24 to 46 hours, which justified the conclusion that the increases in amino nitrogen observed when egg albumen or enzyme solutions were present could be attributed to the action of the latter.

Erepsin was active upon casein and upon peptone but had no effect upon gelatin.

The use of casein as a substrate by Balls and Swenson can be criticised on the grounds that it fails to distinguish between erepsin-like enzymes and true proteases (e.g. trypsin). The conclusion arrived at by these authors that a "tryptic proteinase" is present in eggs is not justified by their data, if one discounts the reported auxiliary effect of entero-kinase. In some cases no increase occurred on the addition of kinase, in others, slightly higher titration figures were recorded, but considering the limitations of the method these figures do not, to our mind, carry much conviction.

#### *Autolysis Experiments.*

Were a protease responsible for the breakdown of egg albumen, leading to the condition of watery-white, it would be expected that measurements of amino nitrogen in such albumen solutions, suitably buffered, would reveal the presence of such an enzyme especially when incubated at a temperature of 37° for periods ranging from 6 to 24 hours.

It seemed natural to look, in the first place, for such autodigestion of egg albumen alone. Autolytic activity of tissues usually proceeds most rapidly in a slightly acid medium. Tests were however performed with thick albumen solutions buffered at reactions ranging from pH 5.5-6.0; 7.5-8.0. No action was observed at any pH although these eggs were shown to contain an active erepsin as subsequently reported (see Table II, trial 12).

Autolytic proteinases seem, therefore, to be definitely absent from the eggs under consideration in our experiments.

In order to meet the possibility that a proteinase was present but that egg albumen was not a suitable substrate to reveal its activity, experiments were carried out in which egg albumen was added to gelatin solutions buffered at pH 7.8 or 8.4 and with, or without, the addition of kinase. No increase of amino nitrogen could be demonstrated (see trials 1, 9 and 13). The egg concerned in trial 1 was a "watery-white" as understood by the trade.

That the majority of eggs do contain an erepsin-like enzyme particularly associated with the thick albumen was clearly proved by the use of casein or peptone solutions as substrates. The activity is not great and requires a micro-method for its demonstration. The enzyme would appear to be associated in normal eggs with the thick albumen fraction; even in a five-months-old egg a feeble action was found in the thick white but none in the thin. In eggs having floating air cells, this enzyme was demonstrable in both fractions (see trials 6 and 8). In the case of trial 6, the albumens from two eggs were mixed and a greater activity found in the thin white than

the thick. It must be remarked, however, that in one of these eggs only a small quantity of thick white failed to pass the sieve. It is possible that a diffusion of the enzyme had occurred from thick to thin.

It is significant that proteolysis was observed upon peptone and casein as substrates without the addition of enterokinase.

As regards the pH optimum, the preliminary trials Nos. 2-11 appeared to indicate a greater activity at pH 7.8 than at 8.4. It was decided, therefore, to observe the activity of individual albumen solutions, pooled from two eggs, over a sufficiently wide pH range.

These results are recorded in trials 14-16 and Figs. 1, 2, and 3; Fig. 4 representing a composite of the final changes observed in the individual experiments.

It seems clear that two regions of maximum activity exist; the one fairly sharply localised at pH 5.5, the other spreading over the range pH 7.0-8.0. At intermediate reactions a certain amount of activity could still be detected.

It seems to us more likely that two separate enzymes having different optima are concerned, and this suggestion is borne out by the greater relative activity in the pH 7.0-8.0 region in the case represented by Fig. 1, no such difference occurring in the other two cases. It is well known that a mixture of two enzymes having different optima will afford an extended pH-activity curve exhibiting two humps. As an example, the pH-activity curve of pepsin acting upon egg albumen (Northrop 1919) has been selected and reproduced twice upon the same graph paper so that the two curves lie symmetrically about the point of their intersection. This gives a hypothetical case comparable with the conditions obtaining in egg white. It is seen from Fig. 5 that the overlapping results in a composite curve, exhibiting two maxima and an extended intervening range of lesser activity. We consider, therefore, that the curves of Fig. 4 may each be resolved into two partially overlapping components, representing two enzymes whose optima lie at approximately pH 5.5 and 7.0-8.0.

## DISCUSSION.

Several records are to be found in the literature of investigations upon the enzymic activity of eggs, but in many cases the results were not unambiguous.

Wohlgemuth (1905) reported the presence of a proteolytic enzyme in egg-yolk, but was unable to demonstrate any such activity in egg albumen. Koga (1923) denies the presence of a trypsin-like enzyme in yolk, detecting, on the other hand, a not-inconsiderable ereptic activity. Egg white, he considers, contains a fibrinolytic enzyme in small amount. Additional references are quoted by Balls and Swenson.

It is evident from the present work that at least two peptide-splitting enzymes were present in the albumens of the eggs studied, whether normal or "watery-white". The enzyme with a pH optimum about 7.6-8.2 is probably similar to tissue erepsin.

Although our data do not pretend to offer any explanation of the cause of "watery-white" it is of interest to discuss our findings in the light of some of the hypotheses which have been put forward to explain this condition.

In the first place the conclusions of Balls and Swenson are clearly untenable. It is difficult to understand, however, how an ereptic enzyme could in any way modify the condition of the native protein. Our autolysis trials indicate that no breakdown occurs under these conditions at pH values ranging from 5 to 8.

It is also significant that a number of eggs examined within 12 hours of laying have been found by us to exhibit the condition known commercially as "watery-whiteness". Storage is not essential to the development of this condition and in our opinion the condition induced by shaking, as reported by various authors (e.g. Canham 1934, Halnan 1933, Dryden 1934), is not identical with that which appears in certain eggs a few hours after having been laid.

Our observations suggested some association between a tendency towards natural watery-whiteness and imperfect shell formation. In support of this, it may be recorded that imperfectly shelled eggs picked at random from a day's collection proved subsequently, although not in every case, to develop watery-whiteness within a short time after being laid (12 hours), and our experience would indicate that experiments taking cognisance of this fact, might help to throw light upon the problem.

Few, if any, eggs with perfectly developed shells of fine texture and good calcification, result ultimately in watery-whites.

The white of an egg is an elaborately organised structure as may be verified by observing its behaviour when poured upon a sieve. The thick albumen forms a kind of pouch supported no doubt by interlacing fibrillæ of mucin. Within this pouch the bulk of the thin white is contained and is liberated when the structure gives way upon the sieve surface. Even so, both thick and thin fractions contain mucin which becomes visible upon diluting the materials with water.

As stated previously, a low proportion of thick to thin albumen is by no means an invariable accompaniment to the watery-white condition, in fact our experiments have shown that this is rarely the case. The lowered viscosity of the white seems to be due largely to a loss of structural organization. It would be of interest to determine whether the total amount of mucin in watery-white eggs is less than in normal eggs and also what alterations, if any, this protein has undergone.

Artificial disintegration of the albumen, as for example by the injection of trypsin or erepsin into normal eggs, may be expected to result in a lowered viscosity of the white, but this in no way proves that the naturally occurring watery white is produced in the same way.

A hypothesis which makes some appeal to us is that the cause may be sought in an upset of the bound- to the free-water ratio. Proteins are known to combine with a certain proportion of the water in which they are dissolved in such a manner that this quantity of the solvent is bound or eliminated in such a way that it no longer participates in osmotic phenomena. Any release of this bound water causes a diminution in the viscosity of the system. The point is susceptible of experimental investigation and should be considered in any attempt to explain the occurrence of "watery-whites". St. John (1931) has found the bound water in thick egg white to be no less than 26 per cent.

Regarding the whole problem from a detached standpoint, it appears to us that many tacit assumptions have been made which require experimental proof or disproof. Speculation based on false premises is obviously useless. Physico-chemical possibilities have not received the attention they would appear to merit in such a problem. It seems to us that the first point to be elucidated in an approach to the problem is the actual nature of the difference between the colloidal systems of protein in water comprising the total white in normal and watery-white eggs. The function of the mucin appears to be to preserve a structural organization in the white as between the albumen and the other members of the colloidal complex, but how this function is discharged is obscure. Some disorganization seems to take place in "watery-whiteness".

One cannot help recalling the somewhat similar problem of gluten dispersible in water. The protein from a "strong" flour forms a highly viscous colloidal solution, that from a "weak" flour a less viscous one. The reason for this behaviour is not known. Even a highly viscous solution undergoes a more or less rapid spontaneous decrease in viscosity without, as far as can be ascertained, any marked or obvious change in the protein; the action of enzymes has been definitely excluded.\* Our knowledge of protein systems with their possibilities of hydration, dissociation and polymerisation is still so meagre that much work will have to be done before such peculiar changes as that above mentioned can be understood. It is along these lines, however, that we feel the solution of the watery-white problem will ultimately be attained.

#### SUMMARY.

1. The enzymic activity of solutions of thick and thin albumen from normal and watery-white eggs has been studied, using a micro-titration method determining peptide cleavage in terms of the increase in amino nitrogen.

2. Thick or thin albumen alone at pH values ranging from 5.5 to 8.5 suffer no autolysis at 37°, neither can any protein-splitting enzyme be detected at any pH by the addition of gelatin as substrate.

\* Since this was written, an article has appeared by Blagoveschenski and Yurgenson (1935) in which it is claimed that there is present in wheat flour an enzyme which exercises a definite solvent action on these proteins by virtue of a disaggregation rather than an hydrolysis. It is claimed that there is no increase in amino-nitrogen during the process. It would seem highly desirable that egg white be studied in a similar manner.



3. The work of Balls and Swenson is criticised on account of insufficient sensitivity of the method employed and the fact that it is such as would fail to distinguish between an increase in acid groups due to lipolytic and due to proteolytic activity. Moreover, the use of casein as a substrate is incapable of differentiating protease from ereptase activity.

4. The presence in egg white of at least two erepsin-like enzymes having different pH optima (approximately 5.5 and 7.0-8.0) has been demonstrated.

Whether or not these enzymes play any part in the development of "watery-white" is uncertain but for reasons discussed it would seem unlikely.

Some observations upon "watery-white" in eggs are recorded and the problem subjected to a short discussion.

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TABLE I.

Substrate.	Enzyme.	Corresponding Trial No.	pH.	Kinase.	Time.	Increase c.c. N 10 NaOH.	Remarks.
Gelatin 2%.....	—	1	7.8	—	Hours.		
					18 42	Nil Nil	No action.
Casein 5%.....	—	7	7.8	—	2.5 24	Nil Nil	No action.
Peptone 2%...	—	3	7.8	—	1	Nil	No action induced by added kinase.
					46	Nil	
					1	Nil	
					46	Nil -0.02	
Casein 5%.....	Trypsin	—	8.4	—	1 46	+0.07 +0.72	0.2 c.c. extract of fresh pancreas.
Casein 2%.....	Erepsin	—	8.4	—	0.5 18	Nil Nil	No clotting of calcified milk in 18 hours. 1 c.c. extract in intest. mucosa.
Peptone 2%...	Erepsin	—	8.4	—	0.5 18	-0.15 -0.90	Definite action on casein at 7.8.
Gelatin 2%...	Erepsin	—	8.4	—	0.5 18	-0.20 -1.05	Definite action on peptone at pH 8.4.
Gelatin 2%...	Erepsin	—	8.4	—	0.5 18	Nil +0.05	No action.

TABLE II.

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
1	Gelatin 2%	1	2 days	Thick	47.7	8.4	—	Hours.	—0.35	This egg had a floating air-cell Albumen not centrifuged. Negative results, probably due to bad sampling. 1 c.c. kinase used. No action.
								1	—0.35	
								5	—0.02	
								5	—0.03	
								1	Nil	
2	Casein	3	1 hour	Thick	44.6	8.4	—	5	—0.03	Membranes centrifuged out. 1 c.c. kinase used. No action.
								1	Nil	
								5	—0.02	
								1	—0.02	
								5	—0.02	
3	Peptone 2%	Same three eggs as in experiment No. 2		Thick			—	2	+0.10	Performed day following Experiment No. 2. Albumen solution kept in refrigerator. 2 c.c. kinase used. An ereptase probably present; action increased by kinase. Greater action with thick white.
								21.5	+0.30	
								2	+0.30	
								21.5	+0.43	
								2	+0.02	
4	Peptone 2%	3	1 day	Thick	48.0	7.8	—	21.5	+0.17	An ereptase probably present. 1 c.c. kinase had no auxiliary effect. No action with thin white.
								2	+0.05	
								21.5	+0.19	
								1	+0.13	
								21	+0.25	
				Thin			+	1	+0.12	
								1	+0.22	
								21	Nil	
								1	—0.02	
								21	Nil	
							+	1	+0.04	
								21		

TABLE II. (continued).

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
5	Casein 5%	3	5 months	Thick	41.9	8.4	—	Hours.	—0.10	Feeble action with <i>thick</i> white only.  1 c.c. of kinase used which had no auxiliary effect.
								1.5	—0.15	
								18	+0.05	
								18	Nil	
								1.5	—	
6	Casein 5%	2	4 days	Thin			+	18	—0.15	These eggs had floating air-cells. In one, practically no thick white left. 1 c.c. kinase used. Action greater in the <i>thin</i> white than in the thick.  Kinase had no auxiliary effect.
								18	—	
								1.5	—0.25	
								18	+0.10	
								66	+0.15	
7	Casein 5% Peptone 2%	1	1 hour	Thick	43.4	7.8	+	18	+0.30	No action on peptone at pH 8.4. Definite action on casein at pH 7.8. 2 c.c. kinase used.
								66	+0.40	
								18	+0.18	
								66	+0.36	
								2.5	+0.25	
8	Casein 5% Peptone 2%	1	1 day	Thick	45.2	8.4	—	24	+0.20	This egg had a floating air-cell. Conclusions of former trial confirmed.  2 c.c. kinase used.
								2.5	+0.07	
								24	—0.10	
								2.5	+0.30	
								24	+0.25	
9	Gelatin 2% Casein 2% Peptone 2%	3	1 hour	Thick	53.8	7.8	—	2	—0.02	No action on gelatin or casein at either pH.  Definite action on peptone at pH 7.8.  2 c.c. of kinase used.
								22	—0.04	
								2	—0.09	
								22	Nil	
								2	—0.15	
							+	22	Nil	
								2	—0.07	
								2	+0.05	
								22	+0.03	
								22	+0.25	

## ENZYMIC ACTIVITY OF EGG-WHITE.

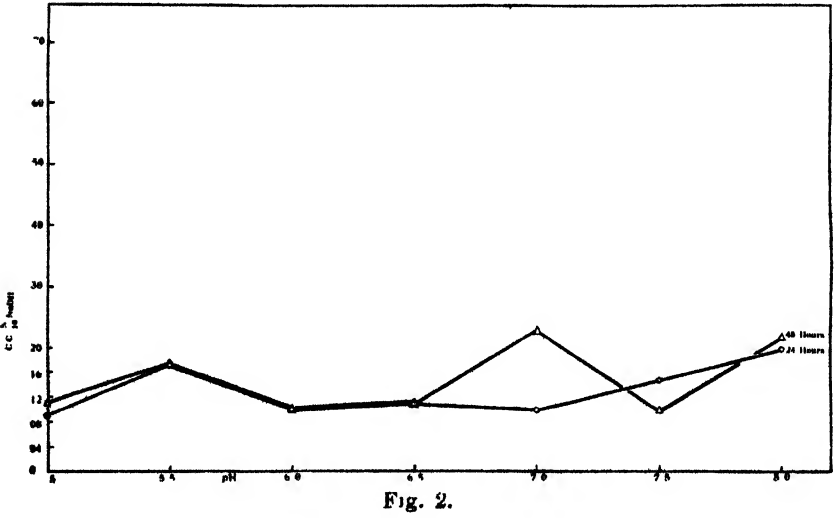
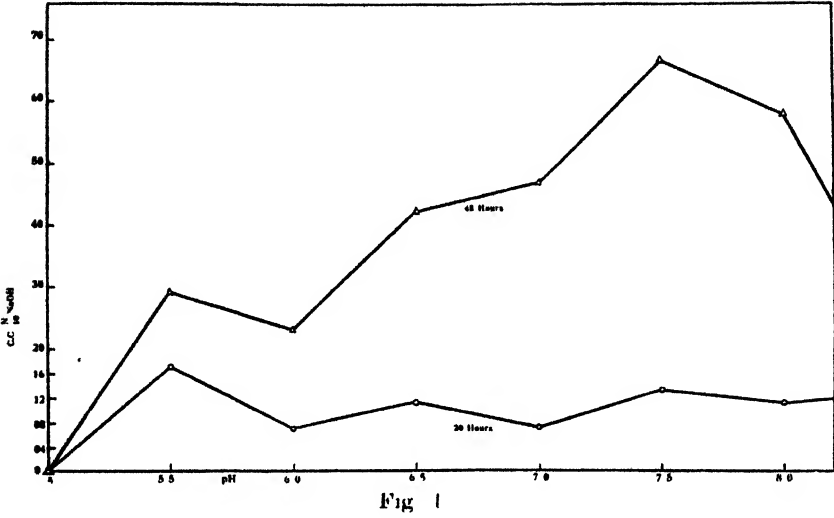
TABLE II. (continued).

Trial No.	Substrate.	Number of eggs.	Age	Kind	% thick white.	pH.	Kinase	Time.	Increase c.c. N/10 NaOH.	Remarks.
10	Casein 2%	2	11 days	Thick	45.0	7.8	—	Hours	Nil	Definite action on both substrates but more marked at the lower pH. Similar results on peptone at pH 7.8 when mucin not removed. 2 c.c. kinase used.
	Peptone 2%								+0.37	
									Nil	
									+0.05	
									+0.15	
									+0.33	
									+0.11	
									+0.23	
11	Casein 2%	2	1 hour	Thick	52.7	7.8	—	Hours	+0.15	Definite action on both substrates in each case. activity appears to be less at the lower pH values 2 c.c. kinase used.
	Peptone 2%								+0.46	
									+0.15	
									+0.35	
									Nil	
									+0.03	
									-0.01	No action at any pH although these eggs known to contain an active enzyme: see previous experiment
									-0.02	
									-0.01	
									-0.01	
									-0.02	There is no evidence of an autolytic protease acting on the albumen as substrate.
									-0.02	
									+0.02	
									+0.03	
									+0.03	
									-0.03	
									Nil	
									-0.04	
									Nil	
									Nil	
13	Gelatin 2%	2	1 hour	Thick	50.0	7.8	—	Hours	Nil	No action on gelatin peptone No kinase used.
	Peptone 2%								+0.08	
									-0.11	
									-0.11	

TABLE II. (*continued*).

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	$\frac{0}{100}$ thick white.	pH.	Kinase.	Time.	Increase cc. N. 10 NaOH.	Remarks.
14	Peptone 2%	2	1 hour	Thick	45.8	5.0		Hours.	Nil	Definite action on peptone: apparently two optima at pH 5.5 and within the range pH 7.0-8.0. (See Fig. 1).  *For pH 8.5 borate buffer used and the figures corrected to the same basis as phosphate buffer.  No kinase used.
						5.5		20	Nil	
						6.0		48	+0.17	
						6.5		20	+0.29	
						7.0		48	+0.07	
						7.5		20	+0.23	
						8.0		48	+0.11	
						8.5		20	+0.42	
						(Borate)		48	+0.07	
								20	+0.47	
15	Peptone 2%	2	1 hour	Thick	53.8	5.0		Hours.	+0.09	Definite action for first 24 hours, little progress after this time. Two optima at pH 5.5 and range pH 7.0-8.0. (See Fig. 2.)  All reagents sterilized by autoclaving and the amount of toluol present increased.  No kinase used.
						5.5		24	+0.11	
						6.0		48	+0.17	
						6.5		24	+0.10	
						7.0		48	+0.10	
						7.5		24	+0.11	
						8.0		48	+0.10	
								24	+0.23	
								48	+0.15	
								24	+0.10	
16	Peptone 2%	2	1 hour	Thick	50.0	5.0		Hours.	+0.02	Definite action on peptone. Two optima at pH 5.5 and range pH 7.0-8.0. (See Fig. 3.)  No kinase used.
						5.5		24	+0.05	
						6.0		48	+0.23	
						6.5		24	+0.31	
						7.0		48	+0.19	
						7.5		24	+0.27	
						8.0		48	+0.13	
								24	+0.25	
								48	+0.23	
								24	+0.23	
						8.0		48	+0.10	
								24	+0.20	
								48	+0.12	
								24	+0.28	

ENZYMIC ACTIVITY OF EGG-WHITE.



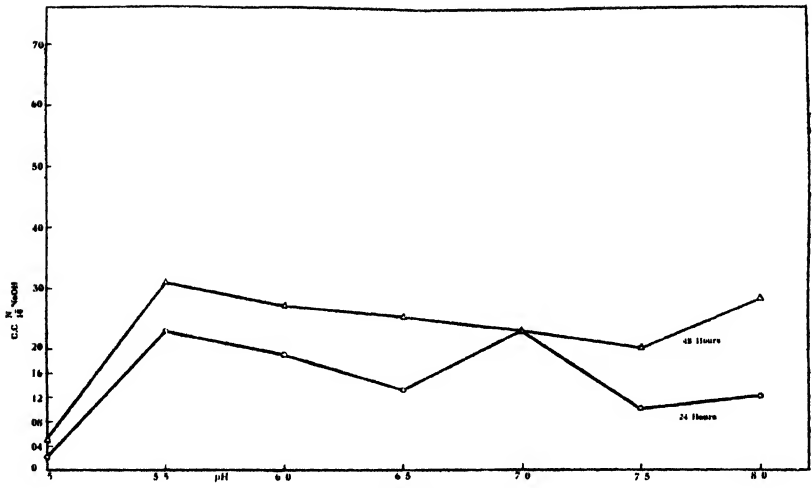


Fig. 3.

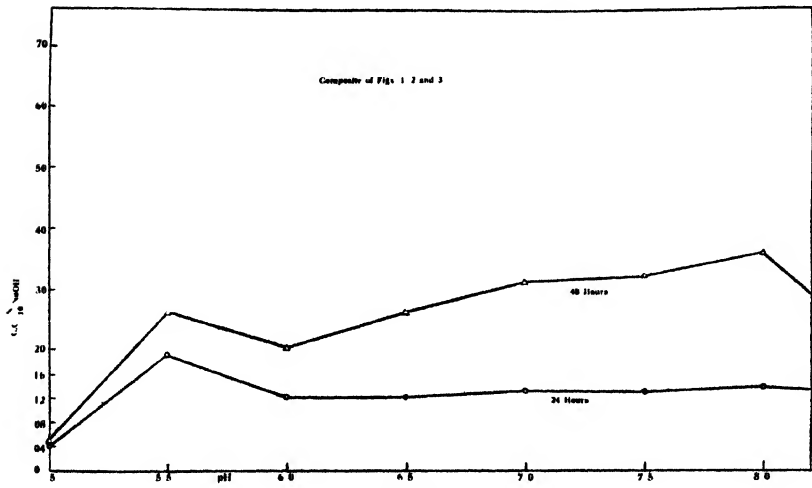


Fig. 4.



ENZYMIC ACTIVITY OF EGG WHITE.

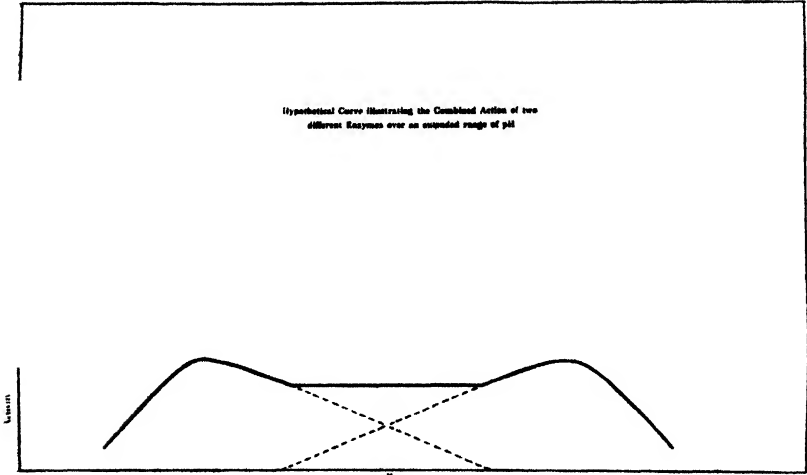


Fig. 5.

## Section XI.

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### Miscellaneous.

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- GREEN, W. J. B. ... Anatomical Studies No. 58. *Diprosopus tetrophthalmus* in a bull calf.
- VILJOEN, N. F., AND DE  
BRUIN, J. H. Anatomical Studies No. 59. On a false masculine hermaphrodite in an avian hybrid.



## **Anatomical Studies No. 58.**

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### **Diprosopus Tetrophthalmus in a Bull Calf.**

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By W. J. B. GREEN, B.V.Sc., Veterinary Research Officer,  
Onderstepoort.

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THE above calf was born in a kaffir location near Maritzburg at the beginning of March, 1934. Unlike most monstrosities of this nature, the calf did not die soon after birth, but was destroyed at 3½ months of age on account of a severe panophthalmia of both central eyes. Otherwise it was in excellent health.

During life the calf was unable to stand or even rise, although on several occasions it made ineffectual attempts to do so—moving around in circles on the ground. The failure to rise was not due to weakness, as it could kick vigorously at the age of two months. This would appear to have been due to the heavy weight of the tremendous head as well as possibly lack of function of the centre of equilibrium. Food could be taken through either mouth, the common method of administration being by bottle to which a long teat was fitted. The sense of hearing was fairly well developed since the head would be slightly raised when approached or spoken to from a distance. Bellowing was heard a few times. When licking, the tip of both tongues would appear simultaneously at the corner of each mouth.

After some weeks and owing to the fact that the eyelids of the central eyes did not function, abrasions appeared on the cornea. These were due to the admittance of foreign particles which irritated the eyes. Infection followed, with the result indicated in the first paragraph.

Post-mortem examination showed nothing unusual except the ophthalmia and the features represented in the accompanying figures (figs. 1-4).

EXPLANATION OF FIGURES



Fig 1 *Diprosopus tetrophthalmus* in a calf.



Fig. 2.—Double tongue of calf.

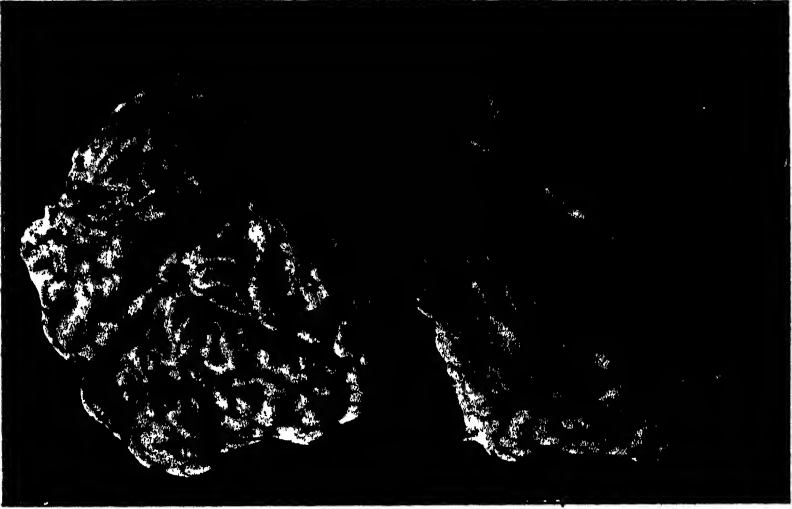


Fig. 3.—Double brain of calf, dorsal view.



Fig. 4.—Double brain of calf, ventral view.



## **Anatomical Studies No. 59.**

### **On a False Masculine Hermaphrodite in an Avian Hybrid.**

By N. F. VILJOEN, M.R.C.V.S., Bloemfontein, and  
J. H. DE BRUIN, University of Pretoria.

WHILE on district duty in November, 1932, in the Lichtenburg District, one of us (N.F.V.) was shown by Mr. Morley, of P.O. Barberspan, a hybrid bird. According to the owner two eggs by a Rhode Island cock out of a guinea-fowl hen were laid some time in 1929. Both eggs hatched, but one chick died. The second chick was successfully reared, and as will be seen in Figs. 1 and 2 the bird was white with numerous red speckled feathers on the back, neck, breast and abdomen. The bird was larger than a Rhode Island hen, but smaller than a cock. The body was much elongated and the head possessed a curved beak which was longer than that of a fowl. The eye was "wild", the comb rudimentary, and the back of the head showed a tuft of small quills which pointed backwards. The wings were well developed and flight was stronger than in the case of the domesticated fowl. The legs appeared to be short for the size of the body.

The habits of the hybrid were always somewhat wild; in fact, it preferred the company of tame guinea-fowls to domesticated fowls. When caught, a weird squawk was emitted and the captor would be readily bitten. Mr. Morley had never noticed any attempt at mating.

Thanks to the owner's generosity, the bird was presented to the Anatomical Department of the Faculty of Veterinary Science at Onderstepoort in December, 1932. Here it was placed in a pen with a White Leghorn cock and fed by the Poultry Officer\* on an egg-producing ration. Although mating took place during the observation period of eighteen months, no eggs were laid.

Finally, on 17th August, 1934, the hybrid was killed and the genitalia examined (J.H.deB). Apart from the fact that the cloaca was smaller than in the case of the usual (egg-laying) hen, all that was seen was a pair of poorly developed testes (T) ( $1.5 \times .6$  cm.), there being no signs of female genitalia or male excretory ducts. (See Fig. 3.)

\* Mr. E. van Manen, to whom thanks are due for his interest in the observation.



With the presence of the male glands and the feminine appearance of the bird, it is evident that the specimen was a false masculine hermaphrodite.

Microscopically the testes were in a condition of aspermatogenesis. (See Fig. 4, A, and Fig. 5, A.) In order to familiarise the reader with the appearance of a normal fowl testicle, a section is shown (Fig. 4, B, and Fig. 5, B) from a year-old White Leghorn cock along with views of *normal* spermatozoa, both kindly stained (Giemsa) by Mr. C. Jackson (Fig. 6, A and B).



Fig. 1.--View of hybrid.



Fig. 2.—Another view of hybrid.



Fig. 3.—Urogenital system of hybrid.



Fig. 4.—A. Testis of hybrid (low power).  
B. Normal cock ( $\times 40$ ).

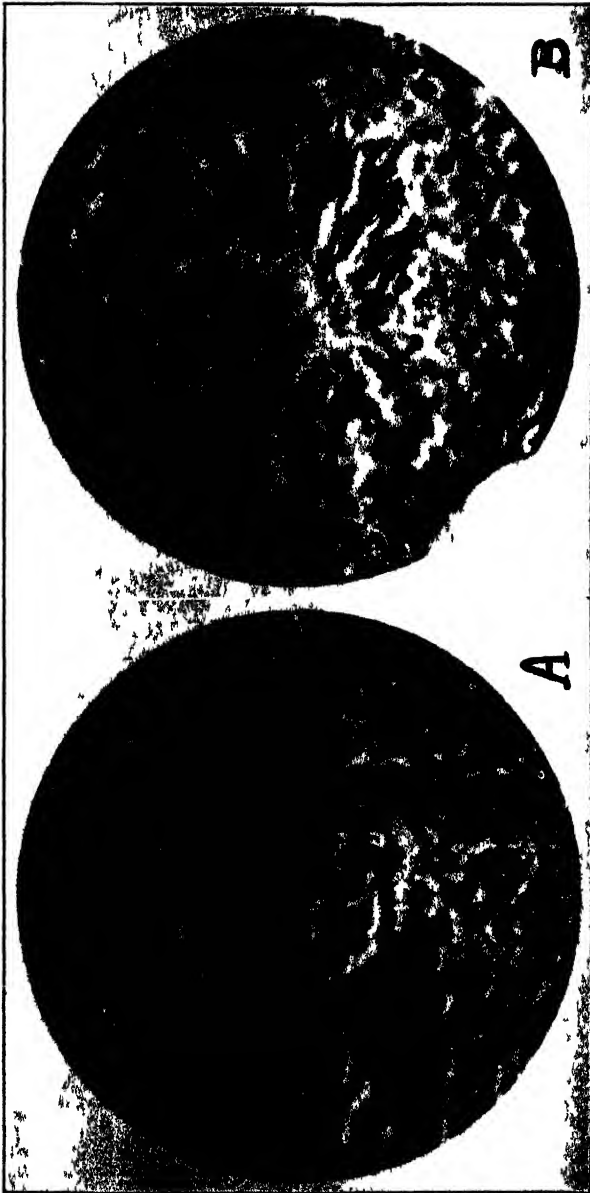


Fig. 5.—A Testis of hybrid (high power)  
B Normal cock (high power) ( $\times 425$ ).

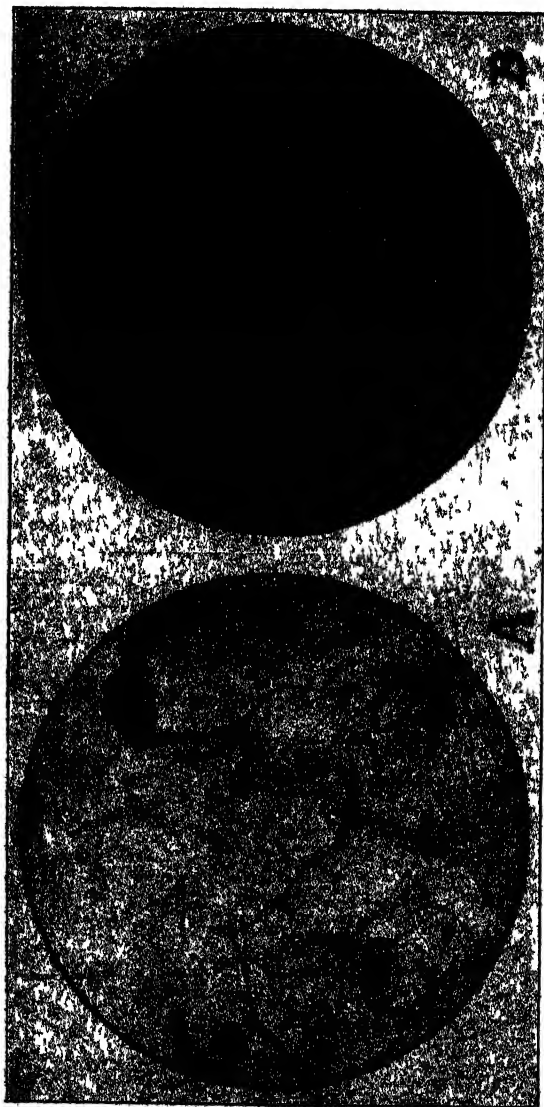


Fig. 6—A. and B Normal spermatozoa of white leghorn cock ( $\times 900$ )



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**PUBLISHED QUARTERLY**

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**Edited by : P. J. DU TOIT, Director**

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OCTOBER, 1935.

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Director of the Onderstepoort Laboratories.**

- Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903-4.\*  
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 Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1905-6.\*  
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 Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.  
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 Eighteenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1932.  
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P. J. DU TOIT,

*Director of Veterinary Services and Animal Industry.*

\* Now out of print.

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## Section I.

# Bacteriology.

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MASON, J. H. . . . .	The Production of Immunity against <i>Cl. welchii</i> , type B, Wilsdon. (The "Lamb Dysentery Bacillus".) . . . . .	391



## The Bloedpens Strain of *Cl. welchii*, Type B, Wilsdon (the "Lamb Dysentery Bacillus").

By J. H. MASON, F.R.C.V.S., F.R.S.E., Empire Marketing Board  
Research Fellow, Onderstepoort.

### INTRODUCTION.

WHEN the writer arrived at Onderstepoort in 1931, he discussed with his colleague, Dr. E. M. Robinson, the similarity of a lamb dysentery-like-disease, "bloedpens", prevalent in certain districts of South Africa, with lamb dysentery as known in Great Britain. From descriptions of the germ isolated, from a naturally-infected lamb, by Dr. Robinson, he came to the conclusion that in all probability, the disease and the causative microbe were the same as in Britain. To confirm this opinion, toxin, prepared from the bloedpens (BP) strain was titrated intradermally (i.d.) in guinea pigs against a lamb dysentery (L.D.) antitoxin brought from overseas (Wellcome Laboratories). It should be emphasised that the only point at issue was whether or not L.D. antitoxin was capable of neutralizing the toxin; no attempt at accurate work was contemplated. Actually 0.01 c.c. of anti-serum neutralized a test dose of toxin containing between 30 and 50 minimum reacting doses (M.R.D.). On this result, it was decided that the toxins of L.D. and BP were serologically very similar and probably identical. The i.d. method of testing was used because, at that time, no mice were available to carry out intravenous (i.v.) titrations. Later, this lack was made good, and a few rough tests were made by the i.v. route, merely to confirm the i.d. results. The apparently discrepant results obtained intravenously initiated this research. It was found that although L.D. antitoxin in a dose of 0.01 c.c. fully neutralized a certain test dose of BP toxin when the i.d. route was used, as much as 0.1 c.c. was unable to neutralize the same test dose by the i.v. route. The probable reason for this is given in the text.

Until the isolation of the "L.D. bacillus" by Gaiger and Dalling in 1922, and its description by Dalling in 1928, only one serological type of *Cl. welchii* was known, the organism originally described by Welch and Nuttall (1892). Bull and Prichett (1917) showed that the toxins of 27 different strains were neutralized by one antitoxin. One strain, *B. Egens*, isolated by Stoddard (1919) was said to be cultural variant; however, Weinberg and Prévot (1927) and Dalling and the writer (unpublished work) showed that serologically it behaved as *Cl. welchii*. Henry (1922) postulated the

presence of 2 toxic fractions which he named a "haemo"- and "myo"-toxin. The writer (unpublished work) was unable to confirm this (by absorption with red cells and muscle) and the results of Dalling *et al.* (1928) and Mason and Glennly (1928) showed that antitoxins of widely different origin all neutralized the toxin of one *Cl. welchii* strain, the titrations being carried out intramuscularly (i.m.) or intravenously (i.v.) in mice or haemolytically against rabbit red cells. Further, the writer has, over a period of 7-8 years, titrated antitoxins of different origin against the toxins of about 6-8 different welch strains; neutralization took place in every instance. Simonds (1915) classified *Cl. welchii* according to its fermentative action on various sugars. Dalling and the writer (unpublished work) received from another laboratory 4 strains classified according to his method; toxins prepared from them were neutralized by an antitoxin prepared against *Cl. welchii*.

The foregoing brief review indicates strongly that the toxins produced by classical *Cl. welchii* are serologically identical. No indication has yet been given that this germ produces more than one toxin, and further, the methods of testing applied (i.v. mouse, i.m. mouse, i.d. guinea pig, and haemolytic) would point to its various actions being due to the same toxic principle. However, in view of the intensive work that is being carried out in various laboratories in different parts of the world on the L.D.—*B. paludis*—*B. ovitoxicus* group of welch-like germs and of the interest that this will stimulate in further investigations into the true welch group, it would occasion no surprise if definite serological variants of *Cl. welchii*, more akin to welch than to L.D., were reported upon in the future.

Apart from some minor cultural variations, the main difference between the L.D. bacillus and *Cl. welchii* was shown to be in their toxins (Dalling, 1928); the L.D. toxic molecule contained some welch toxin as evidenced by the ability of its antitoxin to neutralize welch toxin. Welch antitoxin, on the other hand, had no effect upon L.D. toxin, showing that its toxin contained none of the L.D. fraction. McEwen (1930) described another welch-like germ, *B. paludis*. The toxin of one strain (CT 40) was found by Mason, Ross and Dalling (1931) and by Mason (1933) to be serologically identical with that of L.D.

Bennetts (1932) isolated from the bowel of sheep infected with "Enterotoxaemia", a welch-like germ, *B. ovitoxicus*, and Gill (1933) recovered the same or a very similar organism from the gut of lambs infected with "Pulpy kidney disease". Bennetts stated that *B. ovitoxicus* toxin was neutralized by L.D. and ovitoxicus but not by paludis antitoxins, that welch toxin was neutralized by these three antisera, that L.D. toxin was neutralized by L.D., paludis, but not by ovitoxicus antitoxins and that paludis toxin was neutralized by L.D. and paludis but not by ovitoxicus antitoxin. Gill was able to neutralize the toxin of his strain with one batch of L.D. antitoxin but not with a second batch; the toxins and antitoxins of ovitoxicus and of his germ cross neutralized, whereas paludis antitoxin failed to neutralize either ovitoxicus toxin or that of his bacillus. It should be noted that both the L.D. antitoxins used were prepared under Dalling's supervision at the Wellcome Laboratories. (The reason for

one batch protecting and the other failing to do so will become clear from what follows.) Wilsdon (1931, 1933), examined a considerable number of welch-like germs and was able to group them on the type of toxin they produced. This classification was briefly as follows:—

*Type A* contains one type of toxin (factor W)—classical *Cl. welchii*.

*Type B* contains three types of toxins (factors W, X and Z)—*L.D. bacillus*.

*Type C* contains 2 types of toxin (factors W and Z)—*B. paludis*.

*Type D* contains two types of toxin (factors W and X)—*B. ovitoxicus*.

Further, he showed that this D type and the *B. ovitoxicus* produced maximum toxin after 3-5 days' incubation of the culture at 37° C. Montgomerie and Rowlands (1933) isolated welch-like germs from the intestine of lambs infected with lamb dysentery. The toxins produced therefrom behaved in the following fashion: *paludis* antitoxin neutralized the toxins only when they were produced from a young (18 hours) culture and when the test dose of toxin contained only a few (5) M.L.D.; toxins of 6-30 days' cultures were not affected by it. *Ovitoxicus* antitoxin (Wilsdon Type D) neutralized only when the toxin had origin from an old (6-30 days) culture. One L.D. antiserum (A) neutralized the toxin produced in 18 hours', 6 days' and 30 days' cultures; another L.D. antitoxin (B) behaved in the same way as did the *paludis* serum. (Both these antitoxins were prepared at the Wellcome Laboratories.) A combination of *ovitoxicus* and *paludis* antitoxins neutralized toxins of all ages. The authors conclude that the toxin of the *L.D. bacillus* contains 2 toxic fractions: one, present in high concentration during the early period of incubation and neutralizable by *paludis* antitoxin and the other, present in low concentration during the early states of incubation, but increasing with incubation and neutralizable by *B. ovitoxicus* antitoxin. Dalling (1934), working in collaboration with Montgomerie and Rowlands, examined the L.D. cultures in use in 1932 (these being subcultures of the original sealed-off 1922 culture) and found that these were unable to form the *ovitoxicus* type of toxin, whereas a direct subculture from the 1922 tube produced a filtrate containing both the L.D. and the *ovitoxicus* type of toxin. Further, he states that he examined the antitoxins used by Montgomerie and Rowlands; that (A above) which neutralized their toxins, no matter their age, was of earlier production and that (B above) which neutralized only "young" toxins was of more recent preparation. He concludes that, at some time between 1922 and 1932, the *L.D. bacillus* lost the power to produce the *ovitoxicus* type of toxin, at least, in amounts detectable by the methods employed.

What has immediately preceded explains the apparently discrepant results obtained by Bennetts and Gill; Bennetts obviously received the earlier and Gill the later type of L.D. antitoxin.

[It may be stated, at this point, that Dalling (1932) and the writer [addendum (1) to this paper] have brought forward evidence to show that *B. ovitoxicus* may produce the L.D. type of toxin.]

The final article that need be mentioned is that of Glenný *et al.* (1933). They examined the toxins and antitoxins of the L.D. bacillus, *B. paludis* and *Cl. welchii* and were able to detect 4 toxic fractions in the toxins. These they designated "*alpha*"—the toxin of classical *Cl. welchii* present also in *B. paludis* and the L.D. bacillus (*agni*) filtrates; "*beta*"—a necrosis-producing, non-haemolytic toxin, not present in *welch* but the predominant constituent of L.D. and *paludis* filtrates; "*gamma*"—a constituent of L.D. (*agni*) filtrates, lethal to mice and not neutralized by *alpha* or *beta* antitoxins. Its presence could only be inferred because sera were encountered with a high protective power against L.D. (*agni*) filtrates as judged by the intradermic injection of guinea pigs and low protective power in mice (i.v. inoculation). This toxin is probably contained to a slight extent by *paludis* filtrates; "*delta*"—a haemolysin produced by *B. paludis* and probably to a much less extent by L.D. (*agni*); and "*epsilon*"—a necrosis-producing toxin associated with Wilsdon's Type D (*B. ovitoxicus*) bacillus. This toxin is neutralized by L.D. sera prepared prior to 1930, but not by recent batches.

A brief summary of the results of research on the toxins of the Welch-L.D.-*paludis*-*ovitoxicus* group is as follows. There are three main toxins produced (following Glenný's nomenclature):—"*alpha*" produced by all members, "*beta*" produced by L.D. *paludis*, and according to Dalling and the writer's earlier results to a slight extent by *ovitoxicus* but not by classical *Cl. welchii* and "*epsilon*", produced by the original L.D. strain and by *B. ovitoxicus* but not by *B. paludis* or *Cl. welchii*. "*Gamma*" is a lethal but non-necrotic fraction produced by L.D. and probably to a slight extent by *paludis* and "*delta*" is a haemolysin formed by *paludis* and to a slight extent by L.D.

## METHODS.

### TOXIN PRODUCTION.

Robertson's meat broth was used in all instances, being thoroughly boiled and rapidly cooled just prior to inoculation. After 18 hours' or 5 days' incubation at 37° C., the culture was passed through paper pulp and then through a Berkefeld candle, and if the toxin was to be used in the liquid state was preserved by adding enough phenol-ether (equal parts) to make a 1.0 per cent. concentration. Dry stable toxin was obtained by saturating the toxic filtrate with ammon. sulph., pressing the precipitate, and drying it *in vacuo* over  $H_2SO_4$ . For use, 100 mgm. of dry toxin was dissolved in 5.0 c.c. of saline.

### TOXOID PRODUCTION.

Enough formalin (40 per cent. formaldehyde) was added, either to the whole culture (freed from meat particles) or to the toxic filtrate, brought to pH 7.4, to make a 0.4 per cent. concentration and the formalised material incubated at 37° C. until more than 0.25 c.c. (injected i.v.) was required to kill a mouse. This usually took from 3 to 7 days.

## ANTITOXIN PRODUCTION.

Goats were used throughout, immunization commencing with the subcutaneous injection of formol-toxoid and finishing with unmodified toxin.

## NEUTRALIZATION TESTS.

Toxin and antitoxin were mixed, allowed to stand for one hour at room temperature (18°-26° C.) and injected i.v. into mice. Results were recorded, after 5 days, as "live", "dead" or "symptoms, lived", i.e. showed nervous phenomena but survived the 5-day period. In the intradermic titrations in guinea pigs, the total volume injected was 0.2 c.c. and the results were recorded after 48 hours as + + + + N, + + N, + N, ± N, the "N" referring to the inflammatory necrotic lesion produced. In the haemolytic tests, haemolysin (toxin) and antihæmolysin (antitoxin) were mixed, enough saline added to bring the volume to 1.5 c.c. and after one hour's interval, 1.0 c.c. of a 2.5 per cent. suspension of well-washed rabbit red cells was added. After 2 hours at 37° C. and 2 hours at room temperature, results were recorded as "complete" (C), "partial" (P), "large trace" (L.T.), "trace" (tr), or negative (N) haemolysis.

## EXPERIMENTAL.\*

*Origin of the cultures used:—*

*The bloodpens culture* Isolated from a naturally-infected lamb by Dr. E. M. Robinson.

*Cl. welchii, Type B.* (a) The "1930" variety, brought from the Wellcome Laboratories by the writer. This is a subculture (many generations old) of

(b) The "original" strain, isolated by Dalling and Gaiger in 1922.

*Cl. welchii, Type C* ... McEwen's C.T. 40 strain.

*Cl. welchii, Type D* ... Bennetts' *B. ovitoxicus* (R2), as received from Dr. Bennetts.

## MORPHOLOGY AND CULTURAL CHARACTERS OF THE BLOEDPENS STRAIN.

The bloodpens strain showed no morphological or cultural characteristic that distinguished it from classical *Cl. welchii*. Its fermentative action on the various "sugars" (1.0 per cent. in 1.0 per cent. peptone water) after 14 days' incubation at 37° C. in a MacIntosh and Fildes' jar was as follows: acid and gas formation in saccharose, galactose, glucose, lactose, maltose, dextrine, raffinose, glycerine and inosite; no action on salicine, adonite, mannite and dulcete. Gelatin was liquefied, alkaline egg medium clotted and milk fragmented ("stormy fermentation"). Loeffler's serum medium was partially liquefied, but inspissated horse serum was not attacked.

\* *Cl. welchii* will be referred to as "Type A", the L.D. bacillus as "Type B", *B. paludis* as "Type O", and *B. ovitoxicus* as "Type D". Glenny's nomenclature will be used to designate the toxic fractions.



**BLOEDPENS STRAIN OF "CL. WELCHII", TYPE B, WILSDON.**

*Pathogenicity.*—The intramuscular injection of 0.1 c.c. of an 18 hours' meat broth culture of BP into guinea pigs caused death in from 15 to 18 hours. The post mortem appearance was very similar to that produced by *Cl. welchii*, Type A, to such a degree that the writer could not differentiate, with certainty, those animals killed with the BP organism from those killed with *Cl. welchii*. On a few occasions, the post mortem picture recalled that produced by *Cl. oedematiens*. The germ could be recovered from the heart blood and liver of the animal shortly after death.

**CROSS IMMUNITY TESTS IN GUINEA PIGS.**

(Bloedpens and Type B.)

Formol-toxoids were prepared from the BP and Type B strains. The latter germ was of the 1930 variety and thus incapable of forming the epsilon toxic fraction.

*BP Toxoid Immunized Guinea Pigs.*—These received two subcutaneous injections of 2.0 c.c. of toxoid at three weeks' interval, and were tested for immunity thirteen days after the second inoculation by the cumulative M.L.D. method (see Mason, Ross and Dalling, 1931). Table 1 records the results.

TABLE 1.

*Amount of Toxin tolerated by G.Ps. Immunized with BP Toxoid.*

*Test Toxin.*

(M.L.D. withstood.)

G.P.	BP 1.	Type B, 1930, 1.
1.....	1-4	
2.....	1-4	
3.....	16	
4.....	32-64	
5.....		1-4
6.....		8-16
7.....		>64

[The "1" of BP 1 and Type B 1930, 1 means that the toxin was obtained from a culture incubated for one day (18 hours). The toxoid used to produce immunity was made from a toxin of a one-day culture.]

*Type B, 1930 Toxoid Immunized Guinea Pigs.*—The toxoid was prepared from a one-day toxin. The injections, the test toxins and the method of test were the same as noted for "BP" toxoid immunized guinea pigs. Table 2 gives the results,

A further test was carried out on two guinea pigs which had received two injections of 2·0 c.c. each of Type B, 1930 toxoid and had survived the intravenous administration of 12 M.L.D. of toxin. One week later, they survived the i.v. injection of 16 but not 32 lethal doses of BP 1 toxin.

TABLE 2.

*Amount of Toxin tolerated by G.Ps. Immunized with Type B, 1930, 1 Toxoid.*

*Test Toxin.*  
(M.L.D. withstood.)

G.P.	BP 1.	Type B, 1930, 1.
1.....	<1	
2.....	<1	
3.....	1-4	
4.....	4-8	
5.....	8-16	
6.....	16-32	
7.....		1-4
8.....		16-32
9.....		>32
10.....		>32
11.....		>32

The results presented in tables 1 and 2 show that the two toxins have at least one important antigen in common. The anti-toxin binding values of the two test toxins used to estimate the degree of immunity produced cannot be compared, since, as will be shown later, they differ qualitatively.

#### THE TOXIN PRODUCED BY THE BLOEDPENS STRAIN.

##### *The " One-day " Toxin (BP 1).*

A dry toxin was prepared from an 18 hours' culture; the M.L.D. for mice by i.v. injection was 0·001 c.c. From the results obtained in the immunization of guinea pigs (tables 1 and 2), and from a few rough preliminary titrations in mice, it was anticipated that Types B and C antitoxins would neutralize this toxin in multiple proportions. Such was not the case. In titrating Type B toxin (1930 variety) and antitoxin, the result can usually be forecasted with some certainty 2 to 4 hours after the injection of the toxin-antitoxin mixtures. Using a test dose of BP 1 toxin of 0·1 c.c. (100 M.L.D.) the following results were obtained in the titration (mouse i.v.) of two Type B and one Type C antitoxins (table 3).

TABLE 3.

*Titration of a large test dose (0.1 c.c. = 100 M.L.D.)  
of B.P.1 toxin against antitoxins.*

*Antitoxins (cc).*

Type B (1789).	Type B (SC).	Type C (pal).
0.004 ÷ ½ hr.	0.02	0.004 ÷ ½ hr.
0.005 ÷ ½ hr.	0.025 All showing symptoms	0.005 ÷ ½ hr.
0.007 ÷ 3 hr.	0.03 5 hours.	0.007 ÷ 4 hr.
0.01 ÷ o/n	0.04 All dead o/n	0.01 ÷ 5 hr.
0.015 ÷ o/n	0.1	0.1 ÷ o/n
0.1 ÷ o/n	0.25	0.25 ÷ o/n
0.25 ÷ o/n	0.5	0.5 ÷ o/n
0.5 ÷ o/n		

[ ÷ = died; o/n = overnight; hr. = hour(s). Type B (1789) and (SC) were prepared from a "1930" strain.]

The results in table 3 show that such a relatively enormous dose as 0.5 c.c. of Types B and C antitoxins was unable to neutralize the test dose of BP 1 toxin although the appearance of the mice after two or three hours indicated that protection would be afforded. That the antitoxins used were of high value is shown by the fact that relatively small amounts (0.004 c.c.—0.015 c.c.) neutralized 20 to 100 M.L.D. of Type B, 1930 and Type C toxins. However, by reducing the test dose of BP 1 toxin to 0.01 c.c. (10 M.L.D.) full protection was obtained, but 0.05 c.c. was not neutralized by 0.1 c.c. of antitoxin and 0.025 c.c. sometimes was and at other times was not neutralized. Table 4 summarises the results obtained, using a test dose of 0.01 c.c. of BP 1 toxin.

TABLE 4.

*Titration of a Small Test Dose (0.01 c.c. = 10 M.L.D.) of BP 1  
Toxin against Antitoxins.*

*Antitoxins (cc).*

Type B (1789).	Type B (SC).	Type C (pal).
0.0007 ÷ o/n ÷ 2d.	0.0012 ÷ 3h. ÷ 5h.	0.0006 ÷ o/n + 2d. + 3d.
0.0008 ÷ o/n ÷ 3d.	0.0015 /// 4d.	0.0007 ÷ o/n. + 2d.
0.001 /// 4d.	0.002 / 4d.	0.0008 + 2d. / 4d.
0.0012 // 4d.		0.0009 // 4d.

(÷ = died; / 4d. = mouse alive and well on the 4th day; h. = hours.)

Before considering one point of importance in Table 4, the result of determining the M.L.D. of a mixture of 0.1 c.c. of BP 1 toxin and 0.015 c.c. of Type B (SC) antitoxin (theoretically a neutral mixture from the results given in table 4) will be presented. This mixture was set up in a multiple of 5; 0.25 c.c., 0.2 c.c., 0.15 c.c. and 0.1 c.c. killed mice overnight; the mouse receiving 0.075 c.c. was ill after 18 hours, showed "jumpy" symptoms in 3 days but was alive after 6 days. In the titrations recorded in table 4 and in this one, a number of mice did not die until the 2nd or 3rd day, and one mouse showed nervous phenomena not previously seen in titrating Type B toxin and antitoxin. In a further series of tests put up to determine the rise in antitoxin content of the blood of goats immunized with Types B and C toxoids, these nervous phenomena and late deaths were again encountered. It was at this stage that the writer consulted (by letter) Major T. Dalling, who was engaged upon the toxins produced by the original Type B culture and the "1930" subculture of it. He (T.D.) suggested that the BP strain was the same as the original Type B culture and that the inability of the Type B antitoxin to neutralize a large test dose was due to the fact that it contained no epsilon antibody and the toxin did contain a small amount of the epsilon fraction. This point and the relationship of the toxins of BP, Types B, C and D produced from young (18 hours) and old (5 days) cultures was next investigated.

As noted, 0.1 c.c. (100 M.L.D.) of BP 1 toxin was not neutralized by as much as 0.5 c.c. of two "1930" Type B antitoxins and one Type C antitoxin, but 0.01 c.c. was neutralized by quite small amounts. This indicated definitely that, in the BP 1 toxin, there were at least two toxic fractions, one neutralizable by Type B, 1930 antitoxin and another, quantitatively in less amount, not neutralizable by this antitoxin. When a test dose of 0.01 c.c. was employed, this fraction was diluted below one fatal dose and thus was unable to indicate its presence.

The experiments, summarised in table 5, show that, although Type B, 1930 antitoxin was incapable of neutralizing 0.1 c.c. of BP 1 toxin and 0.1 of BP 5 and Type D 1 and 5 antitoxins did not neutralize  $1\frac{1}{2}$  M.L.D. of this toxin, a mixture of Type B, 1930 antitoxin and one of the three mentioned antitoxins did neutralize 0.1 c.c. of BP 1 toxin. (BP 5 and Type D 1 and 5 means that the antitoxins were produced by means of filtrates of 1 or 5 day cultures.)

The results presented in table 5 confirm the view earlier expressed, that BP produces an epsilon and a beta toxic fraction. Further, it is shown that the antitoxins produced with the 5 day BP toxin and the 1 and the 5 day Type D toxins contained so little of the anti-Type B (beta) fraction that 0.1 c.c. was unable to neutralize 1 M.L.D. of Type B, 1930 toxin. However, that Type D can produce, under suitable conditions, some beta toxin will be discussed in an addendum. Further, results, to be recorded later, show that BP 1 antitoxin neutralizes Type D toxin (not neutralizable by Type B, 1930 antitoxin).

TABLE 5.  
*Titration of BP1 Toxin against Antitoxins.*

Toxin (cc).	Antitoxin (cc).				Result.
BP 1.	Type B. 1930.	BP 5.	Type D. 1.	Type D. 5.	
0.1.....	0.1	—	—	—	÷ 36 h.
0.0015.....	—	—	—	0.1	÷ o/n
0.0015.....	—	—	0.1	—	÷ $\frac{1}{2}$ h.
0.0015.....	—	0.1	—	—	÷ o/n
0.1.....	0.1	0.1	—	—	÷ ÷ 36 h.
0.1.....	0.1	0.15	—	—	÷ 3d /4d.
0.1.....	0.1	0.17	—	—	/ 4d.
0.1.....	0.1	0.2	—	—	/ 4d.
0.1.....	0.1	—	0.02	—	÷ ÷ 1d.
0.1.....	0.1	—	0.025	—	// 4d.
0.1.....	0.1	—	—	0.017	÷ o/n ÷ 2d.
0.1.....	0.1	—	—	0.02	// 4d.
Type B. 1930. 0.0005 (1 M.L.D.).....		0.1 ÷	0.1 ÷	0.1 ÷	
Type C. 0.0005 (1 M.L.D.).....		0.1 ÷	0.1 ÷	0.1 ÷	

[÷ = died ; / = lived ; h. = hour(s) ; d. = day(s).]

In table 6, the results of titrating a large (0.1 c.c.) and a small (0.01 c.c.) test dose of BP 1 toxin against BP 1 antitoxin are recorded.

TABLE 6.  
*BP 1 Toxin, Large and Small T.D. versus BP 1 Antitoxin.*

Toxin (cc).	Antitoxin (cc).	Toxin (cc).	Antitoxin (cc).
0.01.....	0.01 ÷ ÷ o/n, ÷ 2, t.		
0.01.....	0.012 ÷ o/n, ÷ ÷ 2,		
0.01.....	0.015 ÷ ÷ ÷ o/n, t.	0.1	0.15 ÷ ÷ ÷ o/n
0.01.....	0.017 ÷ 2, ÷ 4,	0.1	0.17 ÷ o/n, ÷ 4d.
0.01.....	0.02 /// 4.	0.1	0.2 ///

(+ 2 + 4 = died 2, 4 days ; t. = showed nervous "turning" symptoms ; / = lived.)

The results given in table 6 show that the law of multiple proportions is satisfied when BP 1 toxin and antitoxin are titrated together.

In table 7 the amounts of antitoxin necessary to neutralize a test dose of BP 1, Type B, 1930 and Type C toxins are given.

A consideration of the ratios given below table 7 would indicate that the three toxins were detecting the same antitoxins. Some, somewhat minor, discrepancies occur, but the small number of different toxins and antitoxins used does not warrant the statement that they are of real significance.

TABLE 7.

*Amounts of Antitoxin necessary to Neutralize various Toxins by the i.v. Route.*

<i>Antitoxin (cc).</i>				
Toxin (cc).	Type B, 1930 (SC).	Type B, 1930 (1789).	Type C (pal.).	BP 1.
BP 1, 0.01.....	0.0017	0.001	0.0008	0.02
Type B, 1930, 0.005.....	0.003	0.0009	0.001	0.017
Type C, pal, 0.005.....	0.002	0.0006	0.0006	0.01

*Ratios.*

BP 1.....	$(2\frac{1}{8}) \frac{1}{8}$	$(1\frac{1}{4}) \frac{1}{4}$	$(1) \frac{1}{4}$	$(25) 2$
Type B, 1930	$(3) 1\frac{1}{2}$	$(1\frac{3}{4}) 1\frac{1}{2}$	$(1) 1\frac{3}{4}$	$(17) 1\frac{1}{10}$
Type C, pal..	$(3\frac{1}{3}) 1$	$(1) 1$	$(1) 1$	$(16\frac{2}{3}) 1$
	↑	↑	↑	↑

*Titration by the Intradermic Method in Guinea Pigs.*

The minimum reacting doses of the three toxins were as follows: BP 1, 0.001-0.0015 c.c.; Type B, 1930, 0.0005-0.001 c.c.; Type C, pal., 0.0005-0.001 c.c. In table 8 are recorded the amounts of antitoxin necessary to neutralize test doses of the toxins. Table 9 compares the amount of antitoxin required to produce neutralization intradermally and intravenously. The i.d. figures are calculated from those in table 8 and the i.v. figures are those of table 7.

TABLE 8.

*Amounts of Antitoxins required to Neutralize Toxins by the i.d. Route.*

*Antitoxin (cc).*

Toxin (cc).	Type B, 1930 (SC).	Type B, 1930 (1789).	Type C (pal.).	BP 1.
BP 1, 0.01.....	0.012	0.0035	0.0025	0.045
Type B, 1930, 0.025.....	0.01	0.0025	0.002	0.03
Type C, pal., 0.025.....	0.006	0.0015	0.0012	0.0175

*Ratios.*

BP 1.....	→ (5) 2	→ (1½) 2½	→ (1) 2	→ (18) 2½
Type B, 1930	→ (5) 1½	→ (1¼) 1¾	→ (1) 1¾	→ (15) 1¾
Type C, pal..	→ (5) 1	→ (1¼) 1	→ (1) 1	→ (14½) 1
	↑	↑	↑	↑

(The figures under antitoxin represent the amounts of antitoxin required to neutralize the test doses of toxin.)

TABLE 9.

*Comparison of the Neutral Points, obtained by i.v. and i.d. Titration.*

*Toxin (cc).*

Antitoxin (cc).	BP 1.	Type B, 1930.	Type C, pal.
Type B, 1930 (SC)..... i.v. i.d.	0.0017 0.0012	0.003 0.002	0.002 0.0012
Type B, 1930 (1789)..... i.v. i.d.	0.001 0.00035	0.0009 0.0005	0.0006 0.0003
Type C, (pal.)..... i.v. i.d.	0.0008 0.00025	0.001 0.0004	0.0006 0.0002
BP 1..... i.v. i.d.	0.02 0.0045	0.017 0.006	0.01 0.0035

The results given in table 8 show that, by the intradermic method, apparent neutralization of a large test dose (0.1 c.c.) of BP 1 toxin is produced by Type B, 1930 and Type C antitoxins. Further, tests proved that the law of multiple proportions was satisfied when the test dose was raised to 0.2 c.c. or reduced to 0.01 c.c. As 0.025 c.c. of BP 1 toxin plus 0.1 c.c. of Type B, 1930 antitoxin was lethal for mice, it is obvious that 0.1 c.c. of the toxin plus the "1930" variety of Type B antitoxin would contain 4 M.L.D. of free toxin which, from the evidence presented, is epsilon toxin. A comparison was made of the M.L.D. and M.R.D. of three toxins, not neutralizable by Type B, 1930 antitoxin and the antitoxins of which did not neutralize Type B, 1930 toxin, viz., the toxins of BP 5, and Type D, 1 and 5.

These were as follows:—

*Toxin.*

	BP 5.	Type D, 1.	Type D, 5.
M.L.D..... cc	0 0015— 0 002	0 003	0 0003— 0 0005
M.R.D..... cc	0 003	0 015— 0 02	0 0005— 0 0007

It is seen that the M.L.D. and M.R.D. of the two 5-day toxins do not differ greatly, but in the case of the 1-day Type D toxin the difference is between five and seven times. There is the possibility that such a difference holds good for the epsilon fraction of BP 1 toxin. This view is supported by the fact that when a test dose of 0.2 c.c. of BP 1 toxin is titrated, intradermally, against Type B, 1930 antitoxin, "neutral" or "overneutralized" mixtures produce a pale white area on the skin, not seen when the antiserum contains some of the anti-epsilon fraction. These "reactions" simulate those produced by small amounts of Type D toxin, although paler and less marked and would have been recorded as negative unless the skin were closely examined and the point in question were unknown.

Tables 7, 8 and 9 show that it required absolutely less antitoxin to produce "neutralization" i.d. than i.v.; therefore, one may assume the presence in each toxin of a fraction, having a lethal but not a necrotic effect. That it is not epsilon is proved by the fact that neither Type B, 1930, nor Type C toxins contain this fraction. The gamma toxin of Glenny *et al*, answers very well. They found that, with certain sera, as much as ten times more was required to produce neutralization i.v. and i.d. Until evidence to the contrary is produced, it will be assumed that the difference in the i.d. and i.v. titrations is due to the presence of this gamma toxin.

The ratios given under table 8 show that, by the i.d. route, almost complete agreement was obtained in the titration of the toxins and antitoxins.



It need only be mentioned, with regard to the neutralizing power of BP 5, and Type D, 1 and 5 antitoxins against BP 1, Type B 1930 and Type C toxins, that 0.1 c.c. of none was able to neutralize one M.R.D. of any of the three toxins, thus confirming the i.v. results of table 5.

Before proceeding to present the results of the titration of a 5-day BP toxin, those obtained with a 5-day Type B toxin will be given.

### *The "5-day" Type B Toxin.*

#### *A. Type B "1930" Variety and Type C.*

The 5-day toxins produced by these two strains differed from those of one day's incubation only in being weaker, i.e. instead of the M.L.D. being in the region of 0.001 c.c., it was between 0.01 and 0.1 c.c. From 1-10 lethal doses were neutralized by Type B 1930, Type C and BP 1 antitoxins and not by 0.1 c.c. of BP 5 or Type D, 1 and 5 antitoxins.

#### *B. Type B, Original Variety.*

The culture originated from one of a series of tubes, sealed-off by Dalling about 1923 and had not, as had the "1930" variety, been subjected to a series of subcultures. A dry toxin was prepared from a meat broth culture, incubated for five days.

Tests showed that 0.1 c.c. of neither Type B, 1930 nor Type C antitoxins were able to neutralize one M.L.D. (0.005 c.c.) or one M.R.D. (0.06-0.07 c.c.) of the toxin. However, that the brews of toxin used to produce the Type B, "original" antitoxin did contain some beta toxin is shown from the results in table 10.

TABLE 10.

*Amount of Type B, Orig. 5 Antitoxin required for Neutralization.*

		<i>Antitoxin (c.c.).</i>	
<i>Toxin (cc).</i>		<i>Titration i.v.</i>	<i>Titration i.d.</i>
Type B, 1930.....	0.1	0.025	0.01
Type C, <i>pal</i> .....	0.1	0.015	0.008
BP 1.....	0.1	>0.1	0.004**
BP 1.....	0.01	0.0006	

\*\* The reaction produced by 0.1 c.c. of BP 1 toxin plus 0.003 c.c. of Type B, original 5 antitoxin was intense, being that obtained with beta toxin; with 0.004 — 0.006 c.c. of antitoxin, small, white areas were formed, like those previously discussed.

In table 11, the results of titrating various antitoxins against Type B, original 5 toxin are given, and in table 12, the titrating of Type B, original 5 antitoxin against different toxins is recorded,

TABLE 11.

*Amounts of Different Antitoxins required for the Neutralization of Type B, orig. 5 Toxin.*

Toxin (cc).		Antitoxin (cc).
Type B, orig. 5.		
T.D.	i.v. 0.01.....	Type B, orig. 5.
	i.d. 0.1.....	0.1
T.D.	i.v. 0.02.....	BP 1.
	i.d. 0.1.....	0.06 0.04—0.05
T.D.	i.v. 0.025.....	BP 5.
	i.d. 0.1.....	0.05 0.02
T.D.	i.v. 0.05.....	Type D, 1.
	i.d. 0.1.....	0.02 0.025
T.D.	i.v. 0.1.....	Type D, 5.
	i.d. 0.1.....	0.03 0.004
T.D.	i.v. 1 M.L.D.....	Type A, 1.
	i.d. 1 M.R.D.....	>0.1 >0.1

Type B, orig. 5 toxin—M.L.D. —0.005 c.c.

M.R.D. —0.06—0.07 c.c.

(T.D.—test dose.)

Tables 10, 11 and 12 bring out several interesting points. Type B, original 5 antitoxin has a relatively high anti-beta and a low anti-epsilon value—0.025 c.c. and 0.015 c.c. neutralize 0.1 c.c. of Type B, 1930 and Type C toxins respectively, whereas 0.1 c.c. is required to neutralize 1 to 5 M.L.D. of Type D toxin. (It should be noted that the 5-day Type B, original antitoxin was produced by injecting several different brews of 5-day toxin into a goat.) On the other hand, the toxin consists chiefly of the epsilon fraction—0.1 c.c. of neither Type B, 1930 nor Type C antitoxins was able to neutralize 1 M.L.D., whereas 0.02–0.03 c.c. of Type D, 1 and 5 antitoxins neutralized 10–20 M.L.D. This is strong evidence that beta toxin was minimal in amount in Type B, original 5 toxin, since 0.1 c.c. of neither Type D, 1 nor 5 antitoxins neutralized 1 M.L.D. of Type B 1930 toxin (table 5). Little comment can be made on the intradermic titration of Type B, original 5 toxin against the various antitoxins because of the small number of reacting doses in the test dose (slightly more than 1 M.R.D.). It will be noticed that the M.R.D. is about 12–14 times greater than the M.L.D. However, with BP 5 and Type D, 5 toxins, the i.d. test doses of which contained 4 and 12 M.R.D. respectively, much less Type B, original 5 antitoxin was required to neutralize i.d. than i.v.

TABLE 12.

*Amounts of Type B, Orig. 5 AT, required to Neutralize various Toxins.*

Toxin. (cc.)		Antitoxin. (cc). Type B, orig. 5.
BP 1.		
i.v.	0.01.....	0.006
T.D. i.d.	0.1.....	0.004
BP 5.		
i.v.	0.01.....	0.01 — 0.025
T.D. i.d.	0.01.....	0.003—0.004
Type D, 1.		
i.v.	1 M.L.D.....	0.1—0.2
T.D. i.d.	N.D.....	
Type D, 5.		
i.v.	0.0015.....	0.1
T.D. i.d.	0.005.....	0.04
Type A, 1.		
i.v.	0.05.....	0.02
T.D. i.d.	0.05.....	0.02

(T.D.—test dose; N.D.—not done.)

The M.L.D. and M.R.D. of the above toxins were as follows:—

	M.L.D. cc.	M.R.D. cc.
BP 1.....	0.001	0.001
BP 5.....	0.0015—0.002	0.0025—0.003
Type D, 1.....	0.003	0.015—0.02
Type D, 5.....	0.0003—0.0004	0.0007
Type A, 1.....	0.005—0.01	0.005—0.01

One further point requires explanation—0.01-0.025 c.c. of Type B, original 5 antitoxin was required to neutralize 0.01 c.c. (5-6 M.L.D.) of BP 5 toxin; 0.1 c.c. for 0.003 c.c. (1 M.L.D.) of Type D, 1 toxin and 0.1 c.c. for 0.0015 c.c. (12-15 M.L.D.) of Type D, 5 toxin. It has already been shown (table 5) that the antitoxins of these toxins contain no demonstrable anti-beta fraction and it will later be shown that they cross-immunize amongst themselves; therefore, it may be safely assumed that the bulk of their "toxic make-up" is the epsilon fraction. It appeared strange that from 0.01-0.025 c.c. of Type B, original 5 antitoxin should be capable of neutralizing 5-6 M.L.D. of one toxin (BP 5), whereas 0.1 c.c. just neutralized 1 M.L.D. of another (Type D, 1) and 0.1 c.c. 12-15 M.L.D. of a third (Type D, 5). The reason becomes clear if the titration of these toxins against Type D, 5 antitoxin is given (table 13).

It is obvious from table 13 that Type D, 1 toxin contains a large amount of toxoid, inasmuch as it requires 35 times more antitoxin (Type D, 5) to neutralize 1 M.L.D. of its toxin than that of BP 5. In the same way, much more Type B, original 5 antitoxin should be required to produce neutrality; this is borne out when the calculation is made.

TABLE 13.

*Amount of Type D, 5 Antitoxin and Type B, Orig. 5 Antitoxins required to Neutralize various Toxins.*

Toxin cc.	ANTITOXIN CC.					
	Type D, 5.			Type B, orig. 5.		
	T.D. (in MLD.)	Per T.D.	Per MLD.	Ratio.	Per MLD.	Ratio.
BP 5.....	50-60	0.009	0.00018	1	0.0016-4	1
Type D, 5.....	10-15	0.02	0.0013-15	5.6	0.006-8	3 (ab)
Type D, 1.....	7	0.045	0.0064	35	0.1-0.2	25-60

[T.D.—test dose; (ab)—about.]

*The 5-Day Toxin of BP and the 1- and the 5-Day Toxins of Type D.*

The antitoxins of BP 5 and Type D, 1 and 5 did not neutralize 1 M.L.D. or 1 M.R.D. of Type B, 1930 or Type C toxins nor did the antitoxins of the last two neutralize the toxins of the first three. This shows that the beta fraction was absent in the first three and the epsilon in the last two.

TABLE 14.

*Intravenous Titration of Various Toxins and Antitoxins.*

Toxin cc.	ANTITOXIN CC.					M.L.D. of Toxin.
	BP 1.	BP 5.	TB. orig. 5.	Type D 1.	Type D 5.	
BP1 .....T.D.	0.1 0.2	1 MLD >0.1	0.01 0.006	1 MLD >0.1	1 MLD >0.1	0.001-15
BP 5.....T.D.	0.1 0.04	0.1 0.017	0.01 0.01-25	0.1 0.0045	0.1 0.009	0.0015-2
Type B, orig. 5.T.D.	0.02 0.04-5	0.025 0.05	0.01 0.1	0.05 0.02	0.1 0.035	0.005
Type D, 1.....T.D.	0.005 0.1	0.007 0.1	0.003 0.1-0.2	0.01 0.025	0.025 0.045	0.003
Type D, 5.....T.D.	0.001 0.04-5	0.0025 0.08	0.0015 0.1	0.005 0.02-25	0.005 0.017	0.0003-4

(TB orig. 5 = Type B, original 5.)

(The figure opposite T.D., is the test dose used. The figure immediately below it is the amount of antitoxin required to neutralize it.)

Tables 14 and 15 record the amounts of the various antitoxins required to neutralize test doses of the various toxins, by i.v. and i.d. titration.

### Ratios.

The ratios were arrived at by calculating, from table 14, the amount of antitoxin necessary to neutralize the same test dose of toxin for each toxin. BP 1 toxin is not included because, as has previously been shown, it is made up chiefly of beta toxin and therefore not neutralizable by Type B, original 5, or Type D, 1 and 5 antitoxins.

Toxin.	ANTITOXIN.				
	BP 1.	BP 5.	TB. orig. 5.	Type D 1.	Type D 5.
BP 5.....	9	3 $\frac{1}{2}$	22-55	1	2
Type B, orig. 5.....	5-6	5	25	1	$\frac{1}{2}$
Type D, 1.....	8	5 $\frac{3}{4}$	13-26	1	$\frac{1}{4}$
Type D, 5.....	10	8	16	1	$\frac{1}{3}$

All five antitoxins are placed in the same order of potency whatever toxin is used for their titration, with the exception that Type D, 5 antitoxin is a little more than twice as weak when tested against BP 5 toxin as when tested against the other toxins. Other, somewhat minor, disagreements occur—BP 1 antitoxin is stronger by one half to double when titrated against Type B, original 5 toxin than against the other toxins; BP 5 antitoxin is weaker and Type B, original 5 antitoxin is stronger when Type D, 5 toxin is used than is the case with the other toxins. The writer does not feel justified in attempting to offer explanations for differences that may, in large measure, depend upon the avidity of the antitoxins, upon the error of the method of testing or upon factors at present unknown. The definite possibility exists that further research will reveal the presence, in D type filtrates, of toxic fractions, other than the epsilon.

*Intradermic Titrations.*—It must be emphasized, in connection with the i.d. titrations, that much greater difficulty was experienced in obtaining the same results on repeat tests than was the case with the i.v. titrations. In fact, the results noted for Type D, 1 and Type B, original 5 toxins are merely approximations. A probable reason for this is the small number of reacting doses per test dose. Even allowing for the inaccuracy of the i.d. test, it is noticeable that, in every instance, less antitoxin was required to produce "neutrality" i.d. than i.v. Along with this it should be noted that the M.L.D. of each toxin was smaller (by as much as 12-15 times in the case of Type B, original 5) than the M.R.D. These facts point to the presence, in these toxins, of a toxic fraction which is lethal on i.v. injection but which does not produce a skin lesion on i.d. inoculation. The relationship of this to Glenny's gamma toxin has not been fully worked out, but it can be said that it takes the same amount of an epsilon antiserum to produce neutralization (i.v. and i.d.) of an epsilon-containing toxin whether Type B, 1930 antitoxin is added to the mixture or not.

TABLE 15.

*Intradermic Titration of Various Toxins and Antitoxins.*

Toxin cc.	ANTITOXIN CC.					M.R.D. of Toxin.
	BP 1.	BP 5.	TB. orig. 5.	Type D 1.	Type D 5.	
BP 1.....T.D.	0.1 0.045	1 MRD >0.1	0.1 0.004	1 MRD >0.1	1 MRD >0.1	0.001
BP 5.....T.D.	0.1 0.02	0.1 0.01	0.01 0.003	0.1 0.0025	0.1 0.0025	0.0025-3
Type B. orig. 5.T.D.	0.1 0.04	0.1 0.02	0.1 0.01	0.1 0.025	0.1 0.004	0.06-7
Type D, 1.....T.D.	?	0.05 0.01	?	0.1 0.005 ?	0.05 0.001 ?	0.015-2
Type D, 5.....T.D.	0.0025 0.015-2	0.005 0.012	0.005 0.03	0.01 0.017	0.01 0.015-2	0.0007

(The figure opposite T.D. is the test dose used, the figure immediately below it is the amount of antitoxin required to neutralize it.)

*Ratios.*

BP 5.....	8	4	12
Type B. orig. 5	1	1	
Type D, 1.....	2	4	
Type D, 5.....	4	1½	3½

(The ratios were calculated from table 15 in the same way as noted for the ratios under table 14.)

The ratios given under table 15 show no correlation whatever. Disregarding the figures for Type B, original 5 and Type D, 1 toxins, by the use of which consistent results could not be got, and considering only those for BP 5 and Type D, 5 toxins, it is clear that the value to be assigned to the antitoxins varied greatly depending upon the toxin used for the test. One is hesitant in suggesting the presence of still another toxin to explain this discrepancy, but the existence in the toxic filtrates of two fractions and of their antibodies in the antisera, all in different proportions, would meet the facts.

It comes out clearly from the foregoing experiments that the intradermic method is a most unsuitable one for the titration of the toxins or antitoxins of the D type.

## THE TOXIN OF "*CL. WELCHII*", TYPE A, AND ITS RELATION TO THE TOXINS OF "BLOEDPENS", AND "*CL. WELCHII*", TYPES B, C AND D.

It has previously been recorded [Mason, Ross and Dalling (1931), Mason (1933)], that as much as 0·1-0·5 c.c. of potent Type A antitoxin will not neutralize 1 M.L.D. or 1 M.R.D. of either Type B or Type C toxin, whereas Types B and C antitoxins neutralize Type A toxin. Further, Glenny *et al.* (1933) have shown that the anti-alpha titre of a serum bears no relation to its anti-beta titre. Bearing this in mind, no attempt was made to titrate accurately the anti-alpha content of the various anti-toxins—whether or not they did contain alpha antitoxin was investigated.

The results may be briefly recorded as follows: Two potent Type A antitoxins (one made in a goat at Onderstepoort, and the other (G.G. 2748) in a horse at the Wellcome Laboratories) were unable, in a dose of 0·1 c.c.-0·2 c.c. to neutralize 1 M.L.D. or 1 M.R.D. of Type B 1930, Type C, Type B, original 5, BP 1, BP 5, Type D 1 and 5 toxins. On the other hand, from 0·01-0·02 c.c. of the antitoxins of these toxins neutralized from 5-15 M.L.D. of a Type A toxin.

### *Haemolysis.*

It is well-known that *Cl. welchii* produces a haemolysin, and Glenny *et al.* (1933) recorded experiments dealing with that of Types B and C.

Table 16 summarises the results obtained in the titration of a number of toxins and antitoxins for haemolytic and anti-haemolytic power respectively.

Little comment need be passed on the data in Table 16. From the ratios, one may conclude that the haemolysins, contained in the various toxins, are serologically identical. Glenny *et al.* (1933) reported that, in certain Type C filtrates, a haemolysin was present which was not neutralizable by antibody contained in either Types A or B antisera. The Type C toxin used for the above test either did not contain it or if so, in undemonstrable amounts. However, it is realised, that many filtrates, produced under various conditions might have to be prepared before such a haemolysin could be demonstrated.

## ADDENDUM 1.

Dalling (1932) made brief reference to work done by himself and the writer on *Cl. welchii*, Type D (*B. ovitoxicus*, Bennetts). As this work has a strong bearing on what has just preceded, it will not be out of place to record the experiments in somewhat greater detail.

The germ was received from Dr. Bennetts in a sealed-off tube of meat broth, and was labelled "B.L.D. bacillus strain R2 15.8.30". The meat was very fragmented and of a brown pink colour. No disagreeable odour was noticed on opening the tube and Gram-stained smears revealed bacilli of the *Cl. welchii*-type. Attempts by standard methods to demonstrate more than one germ in the culture failed.

TABLE 16.  
Amount of Antihæmolyisin required to Neutralize different Haemolysins.

HÆMOLYSIN.	C.C.		T.A. 748.	T.A. 54.	T.B. 1930.	T.B. orig. 5	T.C. pal.	BP 1.	BP 5.	T.D. 1.	T.D. 5.
	M.H.D.	T.D.									
Type B, 1930.....	0 5	N.D.									
Type B, orig. 5.....	>0 5	N.D.									
Type C, pal.....	0 05	0 2	0 0025	0 0025	0 03	0 02	0 006	0 04	0 1	0 04	0 0015
BP 1.....	0 25	0 5	0 0025		0 02		0 007	0 04	0 08	0 04	0 0015
Type D, 1.....	0 05-1	0 5	0 001		0 01	0 008	0 003	0 02	0 04	0 02	0 0007
BP 5.....	0 5	N.D.									
Type D, 5.....	0 1	N.D.									
Type A, (54).....	0 01	0 1		0 0035	0 04		0 009	0 05	0 15		0 0025
Type A, (318).....	0 003	0 1	0 008		0 08	0 05	0 02	0 15	0 4	0 15	0 005

RATIOS.											
Type C, pal.....		1 $\frac{1}{4}$	1 $\frac{1}{4}$	20	13	4	26	66	26	1	
BP 1.....		1 $\frac{1}{4}$		13		4 $\frac{1}{2}$	26	53	26	1	
Type D, 1.....		1 $\frac{3}{4}$		14	12	4	28	56	28	1	
Type A, (54).....			1 $\frac{1}{2}$	16		4	20	60		1	
Type A, (318).....		1 $\frac{3}{4}$		16	10	4	30	80	30	1	

(M.H.D. = minimal hæmolytic dose; T.D. = test dose; N.D. = not done; T.A. = type A; T.B. = type B, etc.)



The morphological and cultural characteristics were those of *Cl. welchii*, Type A. Glycerine was fermented, Loeffler's serum medium partially liquefied, alkaline egg clotted, and solid serum softened but not liquefied.

*Simple Animal Test.*—The intramuscular injection of 1.0 c.c. of a 24 hours meat broth culture into a guinea pig killed it within 48 hours. The autopsy revealed a pale gelatinous oedema of the subcutaneous tissue, with little or no reddening and reminding one of the picture produced by *Cl. oedematiens*. The small intestines and the adrenals were very congested. A similar post-mortem finding has been obtained in sheep at Onderstepoort.

*The Toxin.*—A dry (ammon. sulph. precipitate) toxin was prepared from a meat broth culture, incubated at 37° C. for 12 hours.

In the tests to be noted, the mice were observed for only 24 hours after the intravenous injection of the toxin-antitoxin mixtures. It was not known, at that time, that epsilon toxin could produce death as long as 5-7 days after injection. Still, it is felt that the results do prove that the Type D antitoxin contained some of the anti-beta fraction and that the Type B antitoxin contained some anti-epsilon. This statement is based upon the fact that the test dose of Type D used (0.1 c.c., 10 M.L.D.) must have consisted, in a preponderating measure, of the epsilon fraction and would certainly have killed a mouse within 24 hours; in the same way, the Type B test dose consisted chiefly of the beta fraction, and contained sufficient M.L.D. (5) to kill a mouse in a few hours. In table 17, cross-neutralization tests between the toxins and antitoxins of Types B and D are recorded. The Type B antitoxin was prepared in a horse by the injection of formol-toxoids and toxins made from cultures, incubated for 18 hours; the Type D antitoxin was made in a rabbit, by injecting into it the formol-toxoid prepared from a 12 hours' culture.

TABLE 17.

*Cross-neutralization Tests between the Toxins and Antitoxins of Types B and D.*

Toxin.	ANTITOXIN (cc.)			
	M.L.D. (cc)		Type B (12479).	Type D.
Type B.....	0.001	Test dose of toxin used (cc)=....	(0.1) 0.003	(0.005) 0.08
Type D.....	0.01	Test dose of toxin used (cc)=....	(0.1) 0.015	(0.05) 0.08

(The non-bracketed figures under antitoxin=c.c. required to neutralize the test doses. The bracketed figures=test doses used.)

From the results given in table 17, the following may be deduced:—

- (1) The Type B antitoxin was produced at a time when the Type B bacillus was still capable of forming the epsilon toxic fraction.
- (2) The toxic filtrate of a 12 hours' Type D culture contained sufficient beta toxin to stimulate the formation of the anti-beta fraction.
- (3) The antitoxin detected in both sera by Type B toxin was the anti-beta portion and by Type D toxin, the anti-epsilon portion.
- (4) Although the Type B toxin used in the above titration may have contained some epsilon toxin and the Type D toxin some beta toxin, such a deduction cannot be made from the data.

The following antitoxins, in amounts of 0·2 c.c. did not neutralize 2 M.L.D. of Type D toxin—*Cl. welchii*, Type A (2853), *Cl. septicum* (2997), *Cl. oedematiens* (2744), *Cl. sordellii*, *Cl. botulinum* (A plus B) and *Cl. histolyticum*. On the other hand, 0·05 c.c. of Type D antitoxin neutralized 7 M.L.D. of a Type A toxin.

The haemolysins of *Cl. welchii*, Types A, B and D proved to be identical serologically.

#### CROSS-IMMUNITY TESTS BETWEEN TYPES B AND D IN GUINEA PIGS.

The formol-toxoids, used to produce the immunity, were made from toxic filtrates of meat broth cultures incubated for 16 hours.

##### *Experiment 1: Type D formol-toxoid.*

13.12.30.—Guinea pigs received 2·0 c.c. of toxoid s.c.

29.12.30.—Guinea pigs received 2·0 c.c. of toxoid s.c.

13. 1.31.—*Test*: 3 Guinea pigs received 1 M.L.D. Type B toxin i.v.—all died.

2·0 c.c. of toxoid injected into the remaining guinea pigs.

20. 1.31.—*Test*: 2 Guinea pigs received 1 M.L.D. Type B toxin i.v.—both died.

##### *Experiment 2: A different batch of Type D Toxoid used.*

20.1.31.—Guinea pigs received 2·0 c.c. of toxoid s.c.

2.2.31.—Guinea pigs received 2·0 c.c. of toxoid s.c.

10.2.31.—Guinea pigs received 2·0 c.c. of toxoid s.c.

16.2.31.—Guinea pigs received 2·0 c.c. of toxoid s.c.

23.2.31.—*Test*:

*Number of M.L.D. of Toxin Tolerated by the Animal.*

G.P.	Type B Toxin.	G.P.	Type D Toxin.
1.....	at least 1	7.....	1-3
2.....	at least 1	8.....	at least 1
3.....	at least 1	9.....	at least 1
4.....	1-3	10.....	at least 1
5.....	1-3		
6.....	1-3		

The results just given show that it is not easy to immunize guinea pigs against Type B toxin when the immunizing agent is Type D toxoid. However, the rather meagre results do not indicate that Type D toxoid conferred a high degree of immunity against the homologous toxin.

*Experiment 3: Type B formol-toxoid.*

A number of guinea pigs were immunized with Type B formol-toxoid (2 s.c. injections of 2·0 c.c., 14 days' interval). When tested with Type B toxin, 10 days after the second inoculation, some withstood 32 M.L.D. These were put aside and, one week later, again received 2·0 c.c. of toxoid. Seven days later, 2 guinea pigs received 1 M.L.D. of Type D toxin, by i.v. injection—both died. In view of this result, two further injections of 3·0 and 4·0 c.c. were given at a week's interval to the four remaining animals. When tested with 1 M.L.D., i.v., one week after the last injection, all died.

One may conclude from this experiment that the Type B toxoid used contained none of the epsilon fraction, or contained it in such small amount that insufficient was present to stimulate the formation of antibodies.

The conclusion to be drawn from the work reported in this addendum is as follows:—

- (1) The Type B toxin used to produce Type B antitoxin 12479 contained a considerable amount of epsilon toxin (0·015 c.c. neutralized 10 M.L.D. of Type D toxin).
- (2) At some time prior to the end of 1930, the Type B bacillus lost the power of forming this toxic fraction (or to form it in anything but minimal amounts), because guinea pigs, highly immune to Type B, did not withstand 1 M.L.D. of Type D.
- (3) Under certain conditions, at present unknown, Type D bacilli are capable of producing the beta toxic fraction. Experiment 2 proves this.

**ADDENDUM 2.**

On reviewing, in 1935, the work just reported in addendum 1, the writer was struck by the fact that the Type D toxin which stimulated the formation of beta antitoxin in a rabbit, was prepared

from a twelve hours' culture. Knowing that the beta toxic fraction is produced early and the epsilon fraction late, in Type B cultures, the possibility existed that very young Type D filtrates might contain sufficient beta toxin to bring about the formation of the antitoxin. On this assumption, goats were immunized with the filtrates of two, four and eight hour cultures. Each animal received 1,500 c.c. of toxin in seven weeks, was then bled and the anti-beta content of its serum ascertained.

The two hour filtrate was relatively non-toxic (M.L.D. mouse i.v. —0.1 c.c.); the four and the eight hour filtrates killed mice in 0.01 c.c. (not tested lower). Type B antitoxin (1930 variety) did not, in a dose of 0.1 c.c. reduce the amount of Type D antitoxin necessary to neutralize any of the three filtrates.

Not one of the three antitoxins prepared with the Type D filtrates, was able, in an amount of 0.2 c.c. to neutralize 1 M.L.D. of Type B, 1930 toxin. The two hour filtrate antitoxin (0.1 c.c.) did not neutralize 1 M.L.D. of Type D toxin, 0.1 c.c. of the eight hour serum neutralized 2 M.L.D. and 0.025 c.c. of the four hour antitoxin neutralized 10 M.L.D.

The writer has had no opportunity of checking up the results noted in addendum 1, but he has no reason to believe that any mistake was made. Therefore, it would appear that the toxin-producing power of *Cl. welchii*, Type D, has undergone a change, inasmuch as it has lost the power to form beta toxin.

## DISCUSSION.

The work detailed in this paper has confirmed many of the findings already reported in the literature. The "bloodpens" bacillus, a germ responsible for lamb dysentery (bloodpens) in South Africa appears to be identical serologically with *Cl. welchii*, Type B (the "lamb dysentery bacillus") as originally isolated by Gaiger and Dalling. It produces two main toxins; young (18 hours) filtrates consist chiefly of Glenny's beta toxin and old (5 days) filtrates of epsilon toxin. Both young and old filtrates contain alpha toxin, and there was evidence that the former contained gamma toxin.

Toxic filtrates of *Cl. welchii*, Type B (1930 variety), and of *Cl. welchii*, Type C (*B. paludis*), were shown to contain beta toxin as their main constituent and alpha as their subsidiary, but were devoid of epsilon. Evidence of the existence of gamma toxin was got but the presence of delta in the Type C filtrate could neither be demonstrated or inferred.

Filtrates of *Cl. welchii*, Type D (*B. ovitoxicus*), both young and old, and of old filtrates of the original Type B strain and of the bloodpens bacillus contained as major toxic constituent the epsilon fraction, alpha being present as subsidiary fraction. The beta toxin was not demonstrated. That gamma was present was possible but was not proved.

The intradermic method proved to be a most unsatisfactory way of titrating toxic filtrates containing, as their chief constituent, the epsilon fraction, or of antitoxins consisting mainly of epsilon antibody. With two toxins this could possibly be accounted for by the small number of reacting doses per test dose. However, with two other toxins, the test doses of which contained a sufficiency of reacting doses, the values to be placed upon antitoxins varied greatly, depending on the toxin used for the titration. Whether or not this has to be accounted for by the existence of two or more serologically different fractions in the toxins and of their antibodies in the antitoxins, all in varying proportions, must await the results of future research. However, owing to the difficulty, experienced throughout all the intradermic work on the titration of toxins and antitoxins containing the epsilon and the anti-epsilon fraction respectively, of obtaining consistent results, the writer feels that the discrepancies may be inherent to the method of the test itself.

Future work will show whether or not a true Type D strain can produce beta toxin. The results given in addendum I prove that on one occasion it did do so. As, however, this was not confirmed some four years later with a subculture of the same strain, final judgment must be withheld until a considerable number of recently isolated organisms has been examined for their toxin-producing power.

Finally, the possibility exists that Type C should not be placed in a separate group. At the present moment, the "1930" variety of Type B and the Type C germ cannot be easily differentiated on the antigenic "make-up" of their toxins. As Type B, 1930 did, at one time, form epsilon toxin, the possibility exists that Type C may also, at one time, have been capable of forming it and has, like Type B, lost this power. However, as many workers throughout the world are at present engaged on the study of the welch group of germs or on disease caused by them, it behoves no one to pass a dogmatic opinion, but rather to await the result of this research.

## CONCLUSIONS.

(1) The "bloedpens" strain of *Cl. welchii*, Type B (the "lamb dysentery bacillus") has been examined. Its toxin has been found to be serologically identical with that of *Cl. welchii*, Type B, as originally described by Dalling.

(2) The main differences and similarities of the toxins of *Cl. welchii*, Type A (classical *Cl. welchii*), Type B, Type C (*B. paludis*) and Type D (*B. ovitoxicus*) have been confirmed.

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## **The Production of Immunity against *Cl. welchii*, Type B, Wilsdon (The "Lamb Dysentery Bacillus").\***

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THE investigation of the disease, lamb dysentery, followed well-established lines—field observation, isolation of the causative germ, reproduction of the disease in normal lambs by the administration of this germ, and, finally, the prevention of the naturally-occurring infection by the use of an antiserum or a prophylactic prepared with this microbe. With a toxin-producing germ, as *Cl. welchii*, Type B, one could aim to produce an antitoxic immunity, an antibacterial immunity or both. In actual field work, no purposeful attempt was ever made to produce an antibacterial immunity in sheep. Doubtless, antibacterial bodies were formed, due to the presence, in the material injected (toxin-antitoxin mixtures, formol-toxoids and anacultures) of autolysed bacteria or of the bacteria, themselves. Whether or not their presence played a useful rôle in the prevention of the disease is not known. As Type B is a toxin-producer, every endeavour was made to devise methods for the obtaining of high antitoxic immunity.

In this communication, the following aspects of the production of immunity will be discussed.

1. The effect on immunity production of:—
  - (a) One and two injections of formol-toxoid (primary and secondary stimulus) and the interval between the injection of the antigen and the test.
  - (b) The amount of formol-toxoid injected.
  - (c) The value (total-antitoxin-binding-value) of the toxoid injected.
2. The immunizing power of small amounts of free toxin.
3. The production of immunity to Type B toxin, with Type A toxoid.
4. Methods of increasing immunity.
5. The production of immunity with Type B bacilli, freed from toxin.

\* The nomenclature suggested by Wilsdon (1931) for the welch group of anaerobes will be used throughout this article.



Dalling and his colleagues (1928) have shown that the immunization of ewes with formol-toxoids or anacultures (formolised whole cultures) of *Cl. welchii*, Type B has reduced the incidence of lamb dysentery in the lambs issue of such ewes. For a number of years, anaculture only has been used for this purpose, chiefly because of the relative ease of production. In brief, its production is as follows:—The meat particles are removed from an 18 hours' meat broth culture of the germ, enough formalin (40 per cent. formaldehyde) to make a 0.4-0.5 per cent. concentration added, the pH adjusted to 7.4 and the material incubated at 37° C. until more than 0.25 c.c. (on intravenous injection) is required to kill a mouse. From 3-10 days at 37° C. are usually required. The value of the toxoid (the word "toxoid" will be used throughout to denote either formol-toxoid or anaculture) is now determined. Varying amounts of toxoid are mixed with 1 unit of a (laboratory) standard antitoxin, and after standing at room temperature for 1 hour, one-half of a test dose of toxin (i.e. one-half of that amount of toxin which is just neutralized by one unit of antitoxin) is added to each mixture. After a further half to one hour at room temperature, the mixtures are injected intravenously into mice. If, for example, the mouse receiving the mixture containing 1/20 c.c. of toxoid, 1 unit of antitoxin and ½ test dose of toxin dies, and that receiving the mixture containing 1/30 c.c. of toxoid lives, 1.0 c.c. of toxoid is said to be equivalent to 15 units of antitoxin, or briefly is of 15 units value.

In testing the immunity produced in animals by toxoids, two methods have been employed:—

(1) *The Cumulative Minimal Lethal Dose (Cumulative M.L.D.) Test* (Mason, Ross, and Dalling, 1931).—This method has given satisfactory results over a period of 7-8 years when applied to guinea-pigs immunized with Type B antigens. Briefly, it is based on the fact that Type B toxin, administered intravenously (i.v.) kills with great rapidity, so that, in the course of 6 hours, 32 or more minimal lethal doses (M.L.D.) may be given in 5 injections (e.g. M.L.D. 1, 3, 4, 8, 16). The result is recorded as "guinea-pig withstood 4-8, etc., M.L.D.", which means that it survived the injection of 4 M.L.D., but not that of a further 4 M.L.D.

(2) *The Titration of the Serum of the Immunized Animal or of the Pooled Sera of a Group of Animals*.—The latter procedure has proved most satisfactory in the writer's hands. In the results to be presented, it will be observed that, by the cumulative M.L.D. test, the individual variation of immunity response is greatly accentuated, some guinea-pigs dying after the injection of 1 M.L.D. and others of the same group withstanding 8 or 16 M.L.D. By testing the pooled sera (i.v. in mice), these individual variations are masked and a valuation figure can be given to the group. Further, the serum can be titrated to about 15-20 per cent. accuracy; such a difference would be difficult to show up by the cumulative M.L.D. method.

Experience over a number of years has shown that a toxoid of 20 or more units usually produces a satisfactory degree of immunity in guinea-pigs.

## EXPERIMENTAL.

## THE EFFECT OF A SINGLE INJECTION OF TOXOID.

The time elapsing between the injection of antigen and the appearance of circulating antitoxin depends, to a large extent, on the value of the antigen. After the injection of 2.0 c.c. of toxoid, value 80 units, into guinea-pigs, antitoxin is demonstrable in 10 days, but whether present before that time has not been ascertained. A low value toxoid of 5-10 units will not stimulate the production of demonstrable antitoxin in 2 up to 4 weeks after injection. However, that it does act as a primary stimulus is shown when a second injection is given 2-4 weeks later; antitoxin is then easily demonstrable 7-12 days after such a secondary stimulus.

In table 1 are recorded the results of testing, by the cumulative M.L.D. method, the immunity produced in guinea-pigs by one subcutaneous injection of a good value toxoid (valued 40 units).

The experiment shows that the maximum immunity is present between the 14th and 21st day after the injection of the antigen, is decreasing at the 28th day and is not demonstrable at the 90th day.

TABLE 1.

*The Effect of a Single Injection of Toxoid.*

G.P.	Cum. M.L.D. test after				
	14 days.	21 days.	28 days.	60 days.	90 days.
1.....	<1	<1	<1	<1	<1
2.....	1-2	<1	<1	<1	<1
3.....	3	1-2	<1	<1	<1
4.....	6-10	1-2	<1	<1	<1
5.....	6-10	2-4	1-2	1-2	<1
6.....	>10	4-8	1-2	4-8	<1

(The guinea-pigs received 2.0 c.c. of the same toxoid s.c., and 6 were removed and tested at the above stated periods.)

*The Effect of Two Injections of Toxoid* (the 2nd injection = "the secondary stimulus").—Again, the value of the toxoid injected determines, to a large extent, the immunity response of the animals. Experiments, planned to show up differences in response of guinea-pigs, which received the secondary stimulus 14, 21, and 28 days respectively after the primary stimulus, did not give a decisive answer, i.e. no significant differences were got. The writer hesitates to stress this point because of the importance that is placed upon a relatively long interval in immunization with diphtheria and tetanus toxoids. Further, in the immunization of sheep, in the field, with Type B toxoid, a long interval, 4-5 months, is allowed, with excellent results, as judged by the immunity of the lambs to lamb dysentery and by the presence of antitoxin in the ewes' sera and colostrum.

*The Effect of the Dose of Antigen.*—As a routine measure, 2·0 c.c. Type B toxoid is given s.c. to guinea-pigs and 5·0 c.c. to sheep. In one experiment, using 2·0 and 0·1 c.c. respectively of toxoid, value 15 units, as primary stimuli and testing, 3 weeks later, the pooled sera of 8 guinea-pigs from each group for its antitoxic power, the following results were got:—

2·0 c.c. guinea-pigs: 0·1 c.c. of serum neutralized 1 M.L.D. of toxin.

0·1 c.c. guinea-pigs: 0·3 c.c. of serum did not neutralize 1 M.L.D. of toxin.

The results of an experiment, planned to test the effect of the size of the secondary stimulus, are noted in table 2. The toxoid was of 25 units value, the primary stimulus was 2·0 c.c. s.c., and the interval between it and the secondary stimulus was 14 days. The immunity test (cumulative M.L.D.) was carried out 10 days after the second injection.

TABLE 2.

*The Effect of Varying the Size (Dose) of the Secondary Stimulus.*

*Test by Cum. M.L.D. Method.*

*Secondary Stimulus c.c.*

G.P.	2·0	G.P.	0·4	G.P.	0·1
1.....	2-4	9.....	< 4	21.....	<2
2.....	8-16	10.....	< 6	22.....	<3
3.....	8-16	11.....	<14	23.....	<3
4.....	16-20	12.....	<14	24.....	<4
5.....	20-30	13.....	<14	25.....	<6
6.....	20-30	14.....	<14	26.....	<6
7.....	>40	15.....	4-8	27.....	4-10
8.....	>64	16.....	4-8	28.....	8-20
		17.....	4-8		
		18.....	8-16		
		19.....	16-32		
		20.....	16-32		

One can say, from the above results, that a 20 times reduction in size of the secondary stimulus results in a decreased immunity response. One would hesitate to say that a 5. times reduction has a definite effect; the indication is that of lessened response, but the experiment would have to be repeated on a large number of animals and the results examined statistically before a final answer could be given.

*The Effect of the Value of the Antigen.*—In table 3 is recorded the immunity produced by 2 injections of toxoids of different value. A similar table could be presented, showing that 1 injection of toxoid (5), value 80 units, so immunized 6 guinea-pigs that they withstood 1-4, 4-8, 4-8, 8, 8-16, and 16-24 M.L.D. of toxin respectively, whereas toxoid (2), value 10 units was incapable of immunizing any one of 6 guinea-pigs to resist 1 M.L.D. of the same toxin.

TABLE 3.  
*The Effect of the Value of the Toxoid Injected.*

<i>Toxoid.</i>				
(1)	(2)	(3)	(4)	(5)
Value (units). 15	Value (units). 10	Value (units). 5	Value (units). 2½	Value (units). 80
<i>Cum. M.L.D. test.</i>				
G.P.	G.P.	G.P.	G.P.	G.P.
1..... <1	1..... <1	1..... <1	1..... <1	1..... 16-32
2..... 4-8	2..... 1-2	2..... <1	2..... <1	2..... >32
3..... 4-8	3..... 2-4	3..... <1	3..... <1	3..... >32
4..... 16	4..... 2-4	4..... <1	4..... <1	4..... >32
5..... >32	5..... 4-8	5..... 4-8	5..... 1-2	5..... >32
		6..... 4-8	6..... 1-2	6..... >32
		7..... 8-16		7..... >32
		8..... 8-16		

[The figures opposite G.P. (guinea pig) 1, 2, etc., are the number of M.L.D. tolerated by the animal.]

Table 3 shows clearly that the antigenic power of a toxoid is in direct relation to its antitoxin binding value. However, one cannot assume, from the above results, that a toxoid of 20 units will be a better antigen than one of 15 units, although one could be almost certain that it would be better than one of 5 units and poorer than one of 80 units. Glenny (1931) discusses the effect, on immunity production, of non-specific material in diphtheria toxoid and suggests that toxoids contain 99 per cent. non-specific and only 1·0 per cent. specific substance. It will readily be appreciated that one lot of toxoid may contain more non-specific material than another and that much of this material may, itself, be antigenic. Again, there is the possibility that the antigen, itself, may vary qualitatively from batch to batch.

The effect of the solubility of the antigen and the route of injection will be discussed under "Methods of Increasing Immunity".

*Unresponsive Animals.*—It will have been noticed, in the results recorded, that the immunity response to the injection of the same toxoid varies enormously in individual guinea-pigs. When this happening was first encountered, it was feared that some guinea-pigs had not been injected and that others had received more than the desired number of inoculations. However, when the phenomenon occurred with every lot of toxoid tested, it was realized that the cause of the apparent discrepancy was the animal itself. To ascertain if the immunity response of the individual was of the same order when two antigens were injected at the same time, the following experiment was carried through. Two groups of guinea-pigs received two injections of Type B toxoid (2·0 c.c.) at 21 days' interval and were tested by the cumulative M.L.D. method 14 days after the secondary stimulus. At the time of the first inoculation, 1·0 c.c. of diphtheria toxoid was injected into one group and 5·0 c.c. into another. Three weeks after this inoculation, each guinea-pig was

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bled and its serum titrated for the presence of diphtheria antitoxin. (The writer is indebted to his former colleague, Mr. A. T. Glenny, for carrying out these tests.) Table 4 records the results.

TABLE 4.

*Comparison of the Immunity Response of Guinea-Pigs Injected at one time with Type B and Diphtheria Toxoids.*

GROUP 1.			GROUP 2.		
2 injections of 2 c.c. Type B toxoid, 1 injection of 1 c.c. dip. toxoid.			2 injections of 2 c.c. Type B toxoid, 1 injection of 5 c.c. dip. toxoid.		
G.P.	Dip. AT.	Type B.	G.P.	Dip. AT.	Type B.
1.....	1/1000	1-4	1.....	1/1000	12-32
2.....	1/10	>32	2.....	1/25	1-4
3.....	1/1000	4-12	3.....	1/25	12-32
			4.....	1/1000	4-8
			5.....	1/1000	1-4
			6.....	1/250	>32

(Dip. AT = diphtheria antitoxin; the figures under Dip. AT. = units; the figures under Type B = the result of the cumulative M.L.D. test.)

Whilst there may be some degree of correlation, the results do not indicate that it is very close.

## METHODS OF INCREASING IMMUNITY.

### (1) THE EFFECT OF MAKING ATOXIC TOXOID SLIGHTLY TOXIC.

(a) To 2.0 c.c. amounts of a rather low value Type B toxoid (11½ units), 10 mouse M.L.D. of Type A, Type B and *Cl. septique* toxins, respectively, were added. Guinea-pigs received 2.0 c.c. s.c. twice (14 days' interval), and were tested (cumulative M.L.D.) 12 days after the secondary stimulus. The results are given in table 5.

TABLE 5.

*The Effect of making Toxoid Slightly Toxic.*

G.P.	Toxoid only.	Toxoid plus			Type B toxin alone 10 M.L.D.
		Type A toxin.	Type B toxin.	<i>Cl. sept.</i> toxin.	
1.....	<1	4-8	<1	<1	<1
2.....	<1	16-24	24-40	<1	<1
3.....	<1	16-24	24-40	8-16	<1
4.....	4-8	24-40	50-60	24-40	<1
5.....	4-8	24-40	50-60		1-2
6.....	16-24		>60		

(*Cl. sept.* = *Cl. septique*; the Type B toxin alone was diluted in broth and 2.0 c.c. injected.)

*Local reactions in above guinea-pigs* (necrosis or evidence of necrosis):—

	14 days after first inoculation.
Toxoid alone.....	0/6
Toxoid plus Type A toxin.....	5/5
Toxoid plus Type B toxin.....	5/6
Toxoid plus <i>Cl. septique</i> toxin.....	4/4
Type B toxin (10 mouse M.L.D.).....	5/5

(5/5 = 5 of 5 animals injected had local reactions.)

(b) An experiment carried out with the same toxoid as in (a) above. The number and the spacing of the injections and the test was the same. The difference was that smaller amounts of toxin were added. The results are given in table 6.

TABLE 6.

*The Effect of making Toxoid Slightly Toxic.*

G.P.	Toxoid only.	Toxoid plus				
		Type A toxin 7 M.L.D.	Type A toxin 3 M.L.D.	Type B toxin 5 M.L.D.	Type B toxin 2 M.L.D.	<i>Cl. sept.</i> toxin 10 M.L.D.
1...	<1	4-8	<1	<1	<1	<1
2...	<1	8-16	<1	8-16	1-4	<1
3...	<1	8-16	<1	16	4-8	1-4
4...	<1	8-16	1-4	16-32	4-8	4-8
5...	<1	8-16	4-8	>32	16-32	4-8
6...	1-4	>32	8-16	>32	16-32	4-8
7...	8-16	>32	8-16	>32	>32	8-16
8...	8-16	>32	>32		>32	
9...	>32	>32	>32			

*Local Reactions in above Guinea-pigs.*

	14 days after first inoculation.
Toxoid alone.....	0/9
Toxoid plus 7 M.L.D. Type A toxin.....	7/9 + 2/9 ? Compare.
Toxoid plus 3 M.L.D. Type A toxin.....	1/9 + 2/9 ?
Toxoid plus 5 M.L.D. Type B toxin.....	1/7 + 1/7 ?
Toxoid plus 2 M.L.D. Type B toxin.....	0/8
Toxoid plus 10 M.L.D. <i>Cl. septique</i> toxin.....	6/7 + 1/7 ?

(7/9, etc. = 7 of 9 guinea-pigs injected showed definite reactions; 2/9?, etc. = 2 of 9 guinea-pigs injected showed very mild reactions; guinea-pigs not accounted for showed no reactions.)

(c) The effect of the addition of toxin to very low value toxoid was investigated. The toxoid used was of 2½ unit value. The procedure was exactly as for (a). Table 7 records the results.

TABLE 7.

*The Effect of making a Low Value Toxoid Slightly Toxic.*

G.P.	Toxoid (2½ units) plus		
	Nil.	Type B toxin 10 M.L.D.	Type A toxin 10 M.L.D.
1.....	<1	<1	<1
2.....	<1	<1	<1
3.....	<1	1-4	<1
4.....	<1	1-4	<1
5.....	<1	8-16	<1
6.....	1-4		<1
7.....	1-4		1-4
8.....			1-4

(d) The effect of a single injection of slightly toxic toxoid was investigated. To 2·0 c.c. amounts of a toxoid, value 25 units, no toxin, 10 mouse i.v. M.L.D. of Type B and 8 mouse i.v. M.L.D. of Type A toxin were added, and injected s.c. into guinea-pigs. Fourteen days later, the cumulative M.L.D. test was applied. The results are given in table 8.

TABLE 8.

*The Effect of a Single Injection of Toxoid made slightly Toxic.*

G.P.	A single injection of toxoid (25 units) plus		
	Nil.	Type B toxin 10 M.L.D.	Type A toxin 8 M.L.D.
1.....	<1	<1	<1
2.....	<1	<1	1-2
3.....	<1	<1	3
4.....	<1	<1	6-10
5.....	<1	1-2	6-10
6.....	<1	1-2	>10
7.....	1-2	1-2	
8.....		3-6	
9.....		6-10	

These four experiments show clearly that amounts of free toxin insufficient, of themselves, to produce demonstrable immunity, are capable, when mixed with toxoid of fair or good value, of improving greatly the antigenic power of the toxoid. However, when added to a very poor toxoid, no significant difference is got. Further, the tests show that only the homologous toxin, Type B, or one having a toxic fraction in common with Type B, viz. Type A, is capable of producing the effect; *Cl. septicæ* toxin was not able to enhance

the value of the antigen. This also shows that the production of a local reaction does not, *per se*, increase the antigenic power of a toxoid.

(2) **THE EFFECT OF ADDING NON-SPECIFIC MATERIAL TO TOXOID.**—The problem of increasing the immunity, produced by diphtheria formol-toxoid, has occupied the attention of many workers, in particular, that of Ramon and his co-workers and Glenny and his colleagues. Ramon (1926) noted the stimulating effect on antitoxin production in the horse when finely ground tapioca was injected with the antigen. The formation of an inflammatory reaction was an accompaniment of the increased immunity. Glenny *et al.* (1926) and Glenny and Waddington (1928) reported upon the increased immunity resulting from the addition of potash-alum to toxoid. Mazzucchi (1929) has recorded the increased protection against anthrax, afforded to animals, when the vaccine is injected along with the irritant glucoside, saponin. To detail all the publications dealing with the non-specific stimulation of immunity would be tedious, but among the substances used are the following: calcium chloride, turpentine, toluol, oil, lanoline, killed germs and cholesterolin.

There are two aspects of the problem (1) the purely scientific, to discover if immunity can be increased with any substance and (2) the practical, to discover those substances, not, in themselves, unduly irritating or toxic which have an activating effect. The second aspect may be further subdivided into (a) the production of hyperimmune sera in horses, where the formation of swellings and abscesses does not cause undue anxiety, (b) the production of immunity in man, and particularly in children, where abscess formation and induration is of serious moment, and (c) the production of immunity in the domestic animals, where reactions are allowable, so long as they are of mild degree. It was with this last aspect that the writer busied himself. Another point, of definite practical importance, had to be kept in mind. The non-specific activator had to be cheap, the preparation of the final product had to be simple, and finally, this product had to be of such consistency that it could be easily injected with a syringe. From the inception of the work, the writer favoured a method which would render the antigen, itself, relatively insoluble. The work of Glenny, Buttle and Stevens (1931) showed that diphtheria toxoid is rapidly eliminated from the body and that the increased immunity resulting from the injection of an alum-precipitated toxoid is due to the slow absorption of the product. For this reason, a number of experiments were carried out to determine the immunizing effect on guinea-pigs of alum precipitates of Type B toxoids.

#### *Experiments with Alum.*

(a) *Yield.*—The most copious yields were obtained when enough alum was added to toxoids to make a 0·5–3·0 per cent. concentration; at 10·0 per cent. it was small and at 0·1 per cent., very small. (NOTE.—In the following discussion the expression “1·0, etc., per cent. alum was added” will mean that enough alum was added to make a 1·0 per cent. concentration.)



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(b) *Value and Nitrogen Content.*—The alum was added to 50 c.c. amounts of toxoid, pH 7.4, in the stated percentages, the precipitate washed twice in distilled water, and the determinations made on the precipitate, dissolved in 2.0 per cent. Rochelle salt. (The writer is indebted to his former colleague, Miss M. Barr, for the nitrogen results.)

Toxoid B2.	Per cent. alum.					
	0.0	1.0	1.5	2.0	2.5	3.0
Value (units).....	12.5	2.5	2.5	5.0	6.0	2.5
Nitrogen (per cent.).....	0.36	0.014	0.046	0.05	0.058	0.036

Toxoid 309.	Per cent. alum.					
	0.0	1.0	1.5	2.0	2.5	3.0
Value (units).....	10.0	2.5	2.5	2.5	2.5	2.5
Nitrogen (per cent.).....	0.38	0.051	0.052	0.046	0.046	0.038

*Immunity Experiment with Toxoid 309.*—The twice washed 1.5 per cent. alum precipitate, resuspended in saline back to the original volume, and the toxoid, itself, were injected, in 2.0 c.c. amounts, s.c. into guinea-pigs. After 21 days, some animals were tested for immunity (cumulative M.L.D.) and the remainder re-inoculated with the antigens. These were tested after a further 10 days. Table 9 records the results.

TABLE 9.  
*Immunizing Power of Alum-Toxoid.*

Toxoid. Immunity after injection.				Alum precipitate. Immunity after injection.			
G.P.	1st	G.P.	2nd	G.P.	1st	G.P.	2nd
1....	<1	13....	<1	5....	<1	19....	< 1
2....	<1	14....	<1	6....	<1	20....	8-16
3....	<1	15....	<1	7....	1-3	21....	8-16
4....	<1	16....	1-4	8....	1-3	22....	8-16
		17....	8-16	9....	1-3	23....	>32
		18....	>32	10....	1-3	24....	>32
				11....	3-6	25....	>32
				12....	3-6		

(Reactions—none of the toxoid guinea-pigs had reactions; all the alum-toxoid animals had small sterile abscesses.)

The indication is strong that, although the alum precipitate was only a quarter of the value of the original, it produced a much higher immunity.

(c) Toxoid, value 15 units, pH 7.4; 0.001 per cent., 0.01 per cent., 0.1 per cent., and 1.0 per cent. alum *added*; the precipitate (unwashed) *plus* the supernatant, injected s.c. into guinea-pigs (2.0 c.c.). Test (cumulative M.L.D.) 14 days after one inoculation and eleven days after two inoculations (21 days' interval). The results are given in Table 10.

(d) As (c), using a toxoid, value  $12\frac{1}{2}$  units, and testing the effect of *adding* 1.0 per cent. alum to it. (The precipitate *plus* the supernatant injected.) Table 11 records the results.

TABLE 10.  
*Immunizing Power of Toxoid Plus Alum.*

G.P.	Alum, per cent.									
	0.0		1.0		0.1		0.01		0.001	
	Immunity after injection.									
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1.....	<1	<1	1-3	<25	<1	< 5	<1	<20	<1	<15
2.....	<1	1-3	<3	>10	<1	<10	<1	<20	<2	<25
3.....	1-2	3-7	<3	>15	<1	<10	<1	<10	<3	<25
4.....		3-7	<3	>20				>15	2-3	>10
5.....		7-9	>6					15	>7	>15
6.....		10-17								>15
7.....		>14								>20

[The (1) and (2) = first and second inoculation.]

TABLE 11.  
*Immunizing Power of Toxoid Plus Alum.*

G.P.	Immunity after one injection of Toxoid plus 1 p.c. alum.	
	Toxoid.	
1.....	<1	<1
2.....	1-2	<1
3.....	2-4	<1
4.....	2-4	<1
5.....	4-8	1-2
6.....		4-8

(e) A toxoid, value  $12\frac{1}{2}$  units, pH 7.4 was treated as follows:—

1. 1.0 per cent. alum added, and the precipitate *plus* the supernatant injected.
2. 1.0 per cent. alum added, the precipitate washed twice in distilled water, resuspended in saline to the original volume and injected.

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3. A 1.0 per cent. alum precipitate washed twice in distilled water, dialysed against distilled water for 4 days at 5° C., suspended in distilled water to the original volume and injected.
4. 1.0 per cent. finely ground tapioca added, and injected.
5. The toxoid, without treatment, injected.

Guinea-pigs received 2.0 c.c. of the above products and, after three weeks, were bled and 0.1 c.c. of their sera titrated against two mouse i.v. M.L.D. of Type B toxin. No serum neutralized this amount. On testing by the cumulative M.L.D. method, the following results were got (Table 12):—

TABLE 12.

*Immunizing Power of Alum Toxoids and of Toxoid Plus Tapioca.*

G.P.	Immunity after one injection of modification.				
	1	2	3	4	Toxoid (no treatment).
1.....	<1	<1	<1	<1	<1
2.....	<1	1-2	<1	<1	1-2
3.....	<1	1-2	<1	1-2	1-2
4.....	<1	1-2	<1	1-2	1-2
5.....	<1	1-2	<1	1-2	1-2
6.....	<1	1-2	1-2	1-2	1-2
7.....	<1		1-2	1-2	1-2
8.....	1-2		2-4	1-2	

(f) The 1.0 and 10.0 per cent. alum precipitates of a toxoid, value 80 units, were washed twice in saline. Their value was less than 2½ units. The immunity test (cumulative M.L.D.) in guinea-pigs, 21 days after a single injection of 2.0 c.c., is recorded in Table 13.

TABLE 13.

*Immunizing Power of 1.0 per cent. and 10.0 per cent. Alum Precipitates.*

G.P.	Immunity after one injection of		
	Toxoid.	1.0 p.c. Alum precipitate.	10.0 p.c. alum precipitate.
1.....	1-4	<1	<1
2.....	4-8	<1	<1
3.....	4-8	<1	<1
4.....	8	1-2	1-2
5.....	16-24	1-2	1-2
6.....	24-40	1-2	1-2
7.....		1-2	4-8

These immunity experiments, planned to show the stimulating effect of an alum precipitate (a precipitate which is only very slightly soluble in water) are very disappointing. The reason would appear

to be the inability of alum to precipitate out more than a fraction of the antigenic material. With toxoid 309 [see under (b)], 1.5 per cent. alum precipitated 50 per cent. of the active material, whereas with the toxoid used in (f) less than 1/30 was obtained in a 1.0 per cent. precipitate. It would appear that with *each* batch of toxoid, a preliminary experiment should be carried out, in which samples are precipitated with 1.0–10.0 per cent. alum and the value and nitrogen content determined. That amount of alum which gives a precipitate, containing the most units per mgm. N should be used, i.e. where the units: mgm. N ratio is high. However, even granting that such a procedure would lead to the obtaining of a better antigen, the results recorded do not indicate that the method has much to recommend it as concerns the increasing of the value of Type B toxoids. In passing, it may be mentioned that the method has proved valuable with toxoids of *Cl. septicum* (writer's unpublished observations).

It should also be noted that the alum precipitates produced local reactions in many of the guinea-pigs injected (abscesses containing a thick pus, usually sterile). No indication was got that animals with such reactions were more immune than those without them.

#### *Experiments with Agar, Saponin and Colloidal Iron.*

These experiments will not be detailed, as no definite indication was got that the addition of the substance to toxoid increased its immunizing value.

(a) *Agar*.—Added, whilst liquid, to toxoid to make 0.1, 0.25 and 0.5 per cent. concentrations. The immunizing power tested on sheep and guinea-pigs; no definite advantage over toxoid.

(b) *Saponin*.—Added to toxoid to make a 0.025–0.05 per cent. concentration. The guinea-pigs injected all developed necrosis (sometimes severe). Results as for agar. These experiments again show that the production of a local reaction did not, *per se*, increase the immunizing value of the toxoid.

(c) *Colloidal Iron*.—Equal parts of toxoid and negatively charged iron (8 mgm. Fe/cc) and positively charged iron (2.4 mgm. Fe/cc.) respectively tested on guinea-pigs. All animals developed large swellings. Results as for agar. (The writer's colleague, Dr. A. I. Malan, kindly prepared the colloidal iron.)

#### *Experiments with Zinc Chloride and the Intraperitoneal Route.*

Alum-precipitates having proved disappointing, the writer looked around for other protein precipitants, the precipitates of which were relatively insoluble in water. Zinc chloride at once suggested itself. (The writer is aware of the use of this substance for the purification of toxoids and toxins, but recalls no published work, in which zinc chloride precipitates have been used for immunization purposes. However, that they have been used for such purposes, he can hardly doubt.) The procedure adopted was similar to that mentioned under "alum precipitation". The  $ZnCl_2$  was added to the toxoid, the precipitate washed twice in water, and resuspended in saline. At the same time, the effect of injecting toxoid intraperitoneally was established.

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*Details of Experiments.*

(a) *Toxoid 7*.—Value 80 units; *ZnCl<sub>2</sub> precipitate*, value 50 units (the precipitate dissolved in 2.0 per cent. sod. citrate and the T.C.P. established; the value is that in terms of the original volume of toxoid precipitated).

*Dose*: 2.0 c.c. *Route*: s.c. and i.p. for toxoid and s.c. for *ZnCl<sub>2</sub> precipitate*. *Test*: the titration of the pooled sera of each group of guinea-pigs against toxin (mouse i.v.) and the cumulative M.L.D. test.

(b) *Toxoid 8*.—Value 80 units; *ZnCl<sub>2</sub> precipitate*, value 50–60 units. Dose and injections as toxoid 7. Cum. M.L.D. test not done.

(c) *Toxoid 9*.—Value 20 units; *ZnCl<sub>2</sub> precipitate*, value 20 units (suspended in saline to half the original volume). *Dose*: 2.0 c.c. *Route*: s.c. only. *Test*: pooled sera only tested.

In the case of toxoid 9, a preliminary experiment was carried out to determine, if possible, the optimum conditions for precipitation with *ZnCl<sub>2</sub>*. The toxoid was precipitated at pH 6 and at pH 7.4 with 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 per cent. *ZnCl<sub>2</sub>*, and the values of the precipitates determined. About 50 per cent. of the antigen was obtained in the 0.5–5.0 per cent. precipitates, and only traces in the 0.1 and 0.25 per cent. precipitates. Further, it seemed as if the 2.5 per cent. yield of the toxoid, pH 7.4, was the least soluble in 2.0 per cent. sod. citrate, but the difference in the solubilities of the various products was very slight.

The tests of the immunity produced were carried out 21 days after the injection of the antigens. Table 14 records the results.

TABLE 14.

*No. Mouse i.v. M.L.D. Neutralized by 0.1 c.c. Pooled Serum.*

	S.C.	I.P.	Zn.
<i>Toxoid 7</i> .....	4–5	40	40
<i>Toxoid 8</i> .....	5	50	12
<i>Toxoid 9</i> .....	? 1		3–4

TOXOID 7.

*Cumulative M.L.D. Test.*

G.P.	S.C.	I.P.	Zn.
1.....	1–4	16–40	16–24
2.....	4–8	>40	24–40
3.....	4–8	>40	24–40
4.....	8		>40
5.....	16–24		>40
6.....	16–24		>40
7.....	24–40		

(S.C. = immunization by subcutaneous route; I.P. = immunization by intraperitoneal route; Zn. = immunization by *ZnCl<sub>2</sub> precipitate*.)

In all three experiments, the  $\text{ZnCl}_2$  precipitates (toxoid 7, 1.0 per cent., toxoid 8, 1.0 per cent., toxoid 9, 2.5 per cent.) proved superior as antigens to the toxoids, themselves. A possible and even probable explanation of the failure of the precipitates of toxoids 8 and 9 to produce the same degree of immunity as that of toxoid 7 is as follows. That of toxoid 7 settled out quickly in saline, was with difficulty dissolved in 2.0 per cent. sod. citrate and produced mild local reactions in guinea-pigs. Those of toxoids 8 and 9 formed colloidal-like suspensions in saline, were easily dissolved in citrate and produced no local reactions. Thus, it would appear that the question is that of solubility; the precipitate of toxoid 7 was very slowly absorbed, high immunity resulting, whilst those of toxoids 8 and 9 were more easily dissolved in the animal body and thus more easily absorbed, producing, as consequence, a lower degree of immunity.

One cannot invoke slow absorption as an explanation for the very high degree of immunity produced by the intraperitoneal injection of toxoids 7 and 8. As Table 14 shows, the immunity was about 10 times greater by this route than by the subcutaneous. Rather would the opposite hold—that the very rapid absorption and “shocking” of the system was the cause. It is well known that this “shock” method answers well in the production of antivirucidal sera and in the later stages of the production of hyperimmune antitoxic sera. However, the writer prefers to present the facts without offering a dogmatic opinion.

#### *Testing of Immunity with Activated Spores.*

The method of preparing the spore suspension is as follows. By culturing *Cl. welchii*, Type B on solid serum for a few days, a suspension, rich in spores, may be obtained. By heating such material at 65° C. for half an hour, most or all of the vegetative elements are killed; the suspension of spores, so produced, has proved stable, in the writer's hands, for at least three months. These spores may or may not, depending upon the number injected, kill a guinea-pig, but 1/100 of a border-line lethal dose will, when “activated”, produce death. The activating agent may be calcium chloride, lactic acid, or glycerine, but, in the author's experience, adrenalin has proved eminently satisfactory. In practice, a dose of spores is added to 0.02 c.c. of 1/1000 adrenalin (as received from the manufacturers) and enough saline added to bring the total volume of fluid to 2.0 c.c. Such, injected i.m. or s.c. into guinea-pigs produces typical gas gangrene. One must exercise the greatest care in working with adrenalin; 0.1 c.c. diluted to 1.0 c.c. with saline will, on injection, produce death with a post mortem picture, simulating that of gas gangrene.

Great use has not been made of this method of testing immunity, but, as will be seen later, guinea-pigs, immune to toxin, resist the injection of activated spore suspension. To dilutions of the stock suspension (held at 5° C.) 0.02 c.c. of adrenalin was added and the total volume of fluid made up to 2.0 c.c. with saline. The dilutions were injected intramuscularly into guinea-pigs. The animals chosen

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for the test were those immunized with toxoid 8 (s.c. injection), toxoid 8 (i.p. injection) and the  $\text{ZnCl}_2$  precipitate of toxoid 8 (s.c. injection). The test was carried out 28 days after the immunizing dose. Table 15 records the results.

TABLE 15.  
*Test of Immunity with Spore Suspension.*

G.P.	Antigen.			
	Toxoid i.p.	Toxoid s.c.	$\text{ZnCl}_2$ ppt.	Dose spores c.c.
1.....	L	L	+ 72 hr.	0.5
2.....	L	L	L	0.5
3.....	L	+ 72 hr.	L	0.25
4.....	L	+ 72 hr.	L	0.25
5.....	L	+ 72 hr.	L	0.1
6.....	L	L	L	0.1
7.....	L	L		0.1

Control G.Ps.	Dose spores c.c.	Result.
1.....	0.01	+ o/n
2.....	0.03	+ 36 hr.
3.....	0.1	+ o/n
4.....	0.25	+ 24 hr.

(L = alive at the end of a week; + = died; hr. = hours; o/n = overnight. Activating dose of adrenalin = 0.02 c.c.; total volume injected = 2.0 c.c.)

The results given above confirm in some measure those of the titration of the antitoxin, as given in Table 14. More guinea-pigs survived the spore injection in the groups whose pooled sera contained the most antitoxin (toxoid i.p. and  $\text{ZnCl}_2$  ppt.). However, it will be noticed that the test is more qualitative than quantitative; three guinea-pigs of the toxoid s.c. group did not withstand 0.25 c.c., 0.25 c.c. and 0.1 c.c. respectively of spores, whereas two others resisted 0.5 c.c. Further, the survivors of all groups had hard, swollen legs, due probably in great measure to the adrenalin.

*Production of Immunity with Heated Toxin and Toxoid.*

*Material Used.*—The toxin from which toxoid 8 was made and the toxoid itself.

*Treatment.*—The toxin boiled half an hour; the toxoid heated at 60° C. for half an hour; the toxoid boiled half an hour.

*Values.*—Toxoid, 80 units; 60° C. toxoid, 40 units; boiled toxoid, <2½ units; boiled toxin, <2½ units.

*Injections.*—Toxoid and 60° C. toxoid—1 injection s.c., test 21 days later; boiled toxin and boiled toxoid—2 injections, 21 days' interval, test 10 days after the 2nd injection.

*Results: Toxoid.*—0.1 c.c. of the pooled sera neutralized 5 mouse i.v. M.L.D. toxin.

60° C. *Toxoid.*—0.1 c.c. of the pooled sera neutralized 1 mouse i.v. M.L.D. of toxin. By the cumulative M.L.D. method, 4 guinea-pigs did not withstand 1 M.L.D. and 3 stood 1—3.

*Boiled Toxin and Toxoid.*—The pooled sera of neither group neutralized 1 mouse i.v. M.L.D. of toxin; no animal withstood 1 M.L.D. of toxin i.v.; the three guinea-pigs of the boiled toxoid group tested did not survive the injection of 0.1 c.c. of activated spore suspension (given in the same way as was noted in Table 15).

These results show clearly that the antigen contained in Type B toxin and toxoid is thermo-labile; it is destroyed on boiling and its value halved when heated at 60° C. for half an hour.

#### PRODUCTION OF IMMUNITY BY THE INJECTION OF SMALL AMOUNTS OF TOXIN.

(1) *Immunity after One Sub-lethal i.v. Dose of Toxin.*—Into seven guinea-pigs, which had received, i.v., from  $\frac{1}{2}$ — $\frac{3}{4}$  of one sure M.L.D. of toxin, was injected, also i.v., one certain fatal dose of the same toxin 15 days later. All died.

#### (2) *Immunity after Several Sub-lethal i.v. Doses of Toxin.*

- (a) A guinea-pig received  $\frac{1}{2}$  M.L.D. of toxin i.v., 5 times within 5 hours. Twenty-four hours later, it withstood 1 sure M.L.D. of toxin i.v.
- (b) As (a), but  $\frac{1}{3}$  M.L.D. 6 times. Withstood 1 M.L.D. 24 hours later.
- (c) As (a), but  $\frac{1}{2}$  M.L.D. 6 times. Did not withstand 1 M.L.D. 24 hours later.
- (d) As (b); withstood  $1\frac{1}{2}$  M.L.D. 24 hours later.
- (e) Five guinea-pigs received  $\frac{1}{2}$  M.L.D. toxin i.v. Tested with 1 M.L.D. after  $\frac{1}{2}$ , 1, 3, 6 and 24 hours—all died.
- (f) A guinea-pig received  $\frac{1}{2}$  M.L.D. 3 times within 2 hours and another  $\frac{1}{2}$  M.L.D. 24 hours later. After a further 24 hours, it survived the injection of 1 sure M.L.D.
- (g) A guinea-pig received  $\frac{1}{3}$  M.L.D. 4 times within 3 hours. It died 15 hours later.

The toxin used in the above experiments was a dry one and the M.L.D. had been established in some dozens of guinea-pigs. Further, the necessary controls were included in each test. One may conclude that a guinea-pig may tolerate a fatal dose of toxin as soon as 24 hours after the i.v. administration of 4–6 sub-lethal doses of the same toxin.



(3) *Immunity after the Intradermic Injection of Small Amounts of Toxin.*

- (a) Two guinea-pigs received 1, 2 and 3 M.R.D. of toxin i.d.—7 days later tested with 1 M.L.D. of toxin i.v., both died.
- (b) Six guinea-pigs received 1, 2 and 3 M.R.D. of toxin i.d., repeated in 7 days. Tested 14 days later with 1 M.L.D. of toxin i.v.—4 died and 2 survived the 1 M.L.D. but not a further 2.
- (c) As (b), but the i.v. test 7 days after the 2nd i.d. injection—none survived 1 M.L.D. i.v.
- (d) As (b), but the i.v. test 2 days after the 2nd i.d. injection—none survived 1 M.L.D. i.v.
- (e) Six guinea-pigs received 1, 2 and 3 M.R.D. toxin i.d., repeated in 14 days. Only one survived 1 M.L.D. of toxin i.v. 14 days later.
- (f) Guinea-pigs received 2·0 c.c. s.c. of Type B toxoid (20 units). Five were tested 14 days later with toxin i.v. *Result:* G.P. (1) <1, (2) 1-3, (3) 1-3, (4) 1-3, (5) 3-7. The 6 animals remaining received 1, 2 and 3 M.R.D. of toxin i.d., and were tested i.v. in a further 2 days. *Result:* G.P. (1) 1-3, (2) 1-3, (3) 1-3, 4 (7-15), (5) 7-15, (6) >15.

Although the injection of a small amount of toxin i.d. produces only a slight degree of immunity, the same amount i.d. acts as a satisfactory secondary stimulus when the primary stimulus was a toxoid of fair value.

IMMUNITY EXPERIMENTS IN GUINEA-PIGS, USING TYPE A AND/OR TYPE B ANTIGENS.

*General Scheme.*—Guinea-pigs received 2·0 c.c. of toxoid, repeated in 14 days, and the test (cumulative M.L.D.) applied 14 days after the 2nd inoculation.

*Experiment 1.*—Two injections of Type A toxoid. *Result:* G.Ps. not immune to 1 M.L.D. of either Type A or B toxin.

*Experiment 2.*—Two injections of Type B toxoid. *Result:* G.Ps. highly immune to Type B but not to Type A toxin.

*Experiment 3.*—First inoculation—Type A toxoid, second inoculation—Type B toxoid. *Result:* G.Ps. not immune to either toxin.

*Experiment 4.*—First inoculation—Type B toxoid, second inoculation—Type A toxoid. *Result:* G.Ps. highly immune to Type B toxin but not immune to Type A toxin.

*Experiment 5.*—First inoculation—Type B toxoid plus 1·0 c.c. of a high value Type B antitoxin, second inoculation, toxoid only.  
*Result:* G.Ps. not immune to Type B toxin.

*Experiment 6.*—As experiment 5, but using a high value Type A antitoxin. *Result:* G.Ps. immune to Type B toxin.

*Experiment 7.*—

- (a) Three injections of Type A toxoid—G.Ps. not immune to 1 M.L.D. of Type A toxin.
- (b) Six injections of Type A toxoid—G.Ps. immune to at least 1 M.L.D. of Type A toxin but not to 1 M.L.D. of Type B toxin.
- (c) Six injections of Type B toxoid—G.Ps. highly immune to Type B toxin and to at least 1 M.L.D. of Type A toxin.

These experiments show that:—

- (1) It is difficult to produce immunity to Type A, even with the use of a Type A antigen.
- (2) Type A toxoid will act as a secondary stimulus to a primary stimulus of Type B toxoid.
- (3) Type A toxoid cannot be used as a primary stimulus (Type B toxoid being the secondary) in the production of immunity to Type B toxin.
- (4) When Type B toxoid (used as a primary stimulus) is "flooded" with homologous antitoxin, no immunity is produced to Type B toxin.
- (5) The "flooding" of a primary stimulus of Type B toxoid with Type A antitoxin does not influence the degree of anti-Type B immunity produced.
- (6) Six injections of Type B toxoid produce immunity to Type A toxin, whereas the same number of injections of Type A toxoid produce no anti-type B immunity.

#### THE PRODUCTION OF IMMUNITY WITH TYPE B BACILLI.

This aspect of the production of immunity has proved the most interesting and most astonishing of all. In comparatively recent times, a considerable amount of work has been carried out to test the immunizing power of the bacilli of the anaerobic group of germs. Green (1929) showed that washed living and washed formalized suspensions of *Cl. chauvæi* and *Cl. septicæ* were capable, on s.c. injection into sheep, of immunizing them against the i.m. inoculation of virulent culture. Robertson and Felix (1930) were able to produce an immune serum in horses by injecting washed and heated *Cl. septicæ* bacilli. This antiserum contained no antitoxin, but protected mice against the injection of washed spores, activated with

calcium chloride. Craddock and Parish (1931) in an attempt to repeat this work found that high value *Cl. septique* antitoxin protected mice against 100 M.L.D. of an activated spore suspension. Henderson (1932) proved that laboratory animals could be immunized against the activated spores of *Cl. chauvæi* by injections of the washed, boiled organism. Later, Henderson (1933) showed that an antiserum, active against the germ, could be prepared by injecting goats with boiled suspensions of the microbe.

The writer has carried the investigation further, and has shown (article in preparation for press) that washed, boiled suspensions of *Cl. chauvæi* immunize sheep against the i.m. injection of culture. These suspensions proved to be excellent immunizing agents and, in controlled experiments, were often superior to anacultures or formol-toxoids. In this investigation, the writer also demonstrated the presence of a toxin in the filtrates of cultures. Such filtrates (or the dialysed ammonium sulphate precipitates) produced reactions when injected i.d. into guinea-pigs or sheep, killed guinea-pigs, mice and sheep on i.v. injection and were specifically neutralizable by antitoxin. Sheep immunized against the toxin (2 s.c. injections of formol-toxoid) developed circulating antitoxin, were immune to culture injected i.m., showed no reaction when toxin was given i.d. and resisted the i.v. administration of a dose of toxin lethal for normal sheep. Sheep hyperimmunized with boiled bacilli (6-8 s.c. injections) were resistant to culture injected i.m., but had no circulating antitoxin and were not immune to the i.d. or i.v. administration of toxin. Further, the injection of boiled formol-toxoid produced a slight degree of immunity, not so high as that evoked by the unboiled material, but sufficient to allow of the animal resisting 1-2 M.L.D. of culture, injected i.m. Such sheep had no circulating antitoxin. One could reasonably conclude that two antigens were involved in the production of immunity to *Cl. chauvæi*, (1) a thermo-stable antigen, producing, probably, antibody only against the bacilli and (2) a thermo-labile antigen (the toxin was destroyed on heating to 60° C. for half an hour) capable of stimulating the formation of antitoxin.

Experiments were planned to prove that the same held good for *Cl. welchii*, Type B. Whilst the presence of a thermo-stable antigen, directed only against the bacilli, has not been disproved, the evidence points to the toxin, or a modification of it, being the chief, and probably the only, antigen connected with the production of immunity to this germ.

*Experiment 1.*—Goats received dense, washed saline suspensions of bacilli, i.v. Some suspensions were living, others heated at 60° C. for half an hour and others boiled for two hours. The sera were destined for agglutinative purposes. Two years later, the sera were titrated against toxin (mouse i.v.) merely to prove that they contained no antitoxin. The astonishing result, recorded in Table 16, was obtained. In the table, the type of germ injected, the number of injections, the length of the course of immunization, whether heated or unheated germs were administered and the titre of the antitoxin in the serum are recorded.

TABLE 16.

*Production of Immunity in Goats by i.v. Injection of Living and/or Killed Type B Bacilli.*

Goat.	Type.	No. injts.	Length of course.	Titre of serum.	
				Mouse M.L.D.	Units.
1.....	B. (S)	14 (545 c.c.)	50 days 285 c.c. boiled bs. 200 c.c. living bs. 60 c.c. 60° C. bs.	266	200
2.....	C. (RS)	5 (215 c.c.)	26 days Living bs. only injected	2,000	1,400
3.....	B. (R)	7 (305 c.c.)	28 days 255 boiled bs. 50 c.c. living bs.	<1	
4.....	C. (R)	7 (170 c.c.)	28 days 155 c.c. boiled bs. 15 c.c. living bs.	<1	

[(S), (RS) and (R) = smooth, rough-smooth and rough variants; under "length of course" the order of the injections is given; under "Mouse M.L.D." the number of mouse i.v. M.L.D. of toxin neutralized by 0.1 c.c. of serum is given; a unit of Type B antitoxin was fixed at the Wellcome Laboratories several years ago.]

Although all the animals received living bacilli, only two produced demonstrable antitoxin. Goat 1 received 3 injections, goat 2, 5 injections, goat 3, 1 injection, and goat 4, 2 injections, of the living germ. The period elapsing after the last administration of the live culture was 7 days in the case of goats 3 and 4, and 21 days for goat 1 (goat 2 received living suspension only). A careful examination of the history of these animals showed that they had been in no other experiment whatsoever. Although the antitoxic titre of their sera had not been ascertained prior to immunization, it was hardly conceivable that it would be in the region of those recorded for goats 1 and 2. To eliminate all sources of error and doubt, a new experiment was commenced.

*Experiment 2.*—A dense suspension of Type B bacilli was washed 5 times in saline. A portion of it was boiled for 2 hours. To a quantity of this boiled suspension, 2 mouse i.v. M.L.D. per 0.5 c.c. were added. The toxicity (mouse i.v.) of the 3 suspensions was now established.

(a) *Living Suspension.*—Mice showed no symptoms after 5 hours when injected with 0.5, 0.25 and 0.1 c.c. i.v.; however, all were dead over-night. In view of the rapidity with which Type B toxin kills, one is justified in concluding that 0.5 c.c. of the material did not contain 1 M.L.D., and that the death of the mice was due to the growth of the germs *in vivo*. An antitoxin binding power test could not be satisfactorily carried out for this reason.

(b) *Boiled Suspension.*—0.5 c.c. was non-toxic, and the value, in terms of antitoxin, was less than 2½ units.

(c) *Boiled Suspension Plus Trace of Toxin*.—0.25 c.c. was toxic and 0.1 c.c. non-toxic. The value was less than 2½ units.

The points that required solution were: (1) will living bacilli, only, stimulate the formation of antitoxin, (2) if so, is this due to the presence of minute amounts of toxin in or adsorbed to the germs, (3) if so, will the presence of small amounts of toxin in a boiled suspension produce the same effect, and (4) will boiled bacilli lead to the production of antitoxin? The results of the experiment are recorded in Table 17. The injections were all made i.v. and just prior to the administration of the antigen, a sample of blood was withdrawn.

Reference to the table shows that 14 days after the commencement of the course, goat 7 (living bacilli) had developed circulating antitoxin, and that the maximum titre was reached on the 19th day. Goat 5 (boiled bacilli) showed immunity on the 19th day, the antitoxin reaching maximum titre on the 23rd day. Goat 6 (boiled bacilli plus toxin) had circulating antitoxin on the 21st day (probably before) but was not followed further because of the result obtained with goat 5.

TABLE 17.

*Production of Immunity in Goats by i.v. Injection of Living and Killed Type B Bacilli.*

Date.	Injection.	No. mouse i.v. M.L.D. neut. by 0.1 c.c. serum.		
		Goat 5. (boiled bacilli.)	Goat 6. (boiled bacilli + toxin.)	Goat 7. (living bacilli.)
27.2.....	5, 6, 7 yes	<1	<1	<1
4.3.....	5, 6, 7 yes	<1	<1	<1
6.3.....	5, 6, 7 yes	<1	<1	<1
8.3.....	5, 6, 7 yes	<1	<1	<1
11.3.....	5, 6, 7 yes	<1	<1	<1
13.3.....	5, 6, 7 yes	<1	<1	4-10
15.3.....	5, 6, yes	2	2	40-100
	7, no			
18.3.....	5, 6, yes	20	ND	200
	7, no			
22.3.....	5, 6, yes	200	20-40*	200
	7, no			
26.3.....	5, 6, yes	200	ND	200
	7, no			

(\* Tested on the 20.3; 5, 6, 7, yes and no = goats 5, 6, or 7 did or did not receive injections. ND = not tested.)

*Experiment 3*.—The sera used in this experiment were prepared in the same way and at the same time as those noted in experiment 1. However, in this case, 2 rough and 2 smooth variants of 3 strains of *Cl. welchii*, Type A were used. The goats received living, boiled and 60° C. heated suspensions. The course of immunization lasted between 3 and 7 weeks. One goat only, that receiving 190 c.c. of a living suspension of an R variant in 16 days, developed antitoxin.

Its serum, in a dose of 0.05 c.c., neutralized 20 M.L.D. of toxin; 0.1 c.c. of the serum of the animal which got the same amount of suspension in the same time, but in this case using the S variant of the same strain, did not neutralize 2 M.L.D. The sera of the other goats, in a dose of 0.1 c.c., did not neutralize 2 M.L.D.

*Experiment 4.*—The immunizing power of one injection of 2.0 c.c. of a dense suspension of washed, boiled Type B bacilli was ascertained.

(a) The bacilli were spun out from an 18 hours' meat broth culture, washed twice in saline and boiled for 2 hours. The value of the suspension was less than  $2\frac{1}{2}$  units. A formol-toxoid, prepared from the filtrate of the same culture was of 80 units value. One group of guinea-pigs received, by s.c. injection, 2.0 c.c. of the boiled organisms and another 2.0 c.c. of the toxoid. After 21 days, the pooled sera from each group was tested for the presence of antitoxin. In addition, the tolerance of the animals in the boiled bacilli group to toxin i.v. was ascertained.

*Result :*

*Boiled Bacilli Group.*—0.1 c.c. of serum neutralized 4-6 mouse i.v. M.L.D. of toxin.

*Cumulative M.L.D. Test.*—G.P. (1) <1, (2) <1, (3) <1, (4) 1-3, (5) 1-3, (6) 3-7, (7) >12, (8) >12.

*Toxoid Group.*—0.1 c.c. of serum neutralized 5-6 mouse i.v. M.L.D. of toxin.

(b) The procedure was exactly as for (a). The bacilli and the toxoid had the same culture as origin. The value of the bacillary suspension was less than  $2\frac{1}{2}$  units, and that of the toxoid was 20 units.

*Result :*

*Boiled Bacilli Group.*—0.1 c.c. of serum neutralized 20-30 mouse i.v. M.L.D. of toxin.

*Toxoid Group.*—0.1 c.c. of serum did not neutralize 1 mouse i.v. M.L.D. of toxin.

*Experiment 5.*—In experiment 2, an attempt was made to immunize goats with washed bacilli, heated for half an hour at 60° C. On two occasions, the i.v. injection of 5.0 c.c. of a dense suspension killed the goats within 18 hours. In the region of the site of inoculation (jugular vein), the muscles were oedematous. Attempts to cultivate *Cl. welchii*, Type B from the oedematous fluid failed. The most probable explanation for the oedema was a leakage of suspension on withdrawing the needle from the vein. This would mean that a minute amount of the material was capable of producing the effect because, on each occasion, after injecting the antigen, 5.0 c.c. of blood was drawn into the syringe and this reinjected before the needle was withdrawn. The toxicity of the suspension for mice (i.v. injection) and guinea-pigs (id. injection) was investigated.

PRODUCTION OF IMMUNITY AGAINST "CL. WELCHII", TYPE B, WILSDON.

*Mice, i.v. Injection.*—0.5 c.c. + at once, 0.25 c.c. + 24 hours, 0.1 c.c. + 24 hours, 0.05 c.c. + 36 hours, 0.025 c.c. + 3 days, 0.01 c.c. L; 2 M.L.D. were not neutralized by 0.1 c.c. of 3 high value Type B antitoxins or by 0.1 c.c. of 3 antisera prepared by injecting goats with living and/or boiled organisms. (+ = died, L = lived.)

*Guinea-pig, i.d. Injection.*—0.025 c.c. produced a small, yellow, circumscribed necrotic area, quite unlike the reaction evoked by the toxin of Type B; 3 M.R.D. were not neutralized by 0.1 c.c. of the sera noted under "mice i.v. injection".

The supernatant of the spun suspension proved non-toxic in a dose of 0.2 c.c., both i.v. and i.d.

These five experiments are of intense practical and scientific interest. To one, like the writer, who has spent years in the preparation of toxins and toxoids for the immunization of horses and sheep, the method offers a probable solution for many difficulties. The antitoxin produced by goat 2, Table (16) in 26 days is as good as the majority produced by horses after a course of injections of toxoid and toxin, lasting 2-3 months. The antitoxins, produced by goats 1, 5 and 7, are of low titre but it has been the writer's experience that the goat does not yield such high value antitoxin as the horse (immunization with toxoids and toxins of *Cl. welchii*, Types A and B, *Cl. septicæ*, and *Cl. botulinum*, Types A, B, C and D). In addition to the speed of immunization, it is possible that the bacilli, obtained from cultures, the filtrates of which were either of high or low value, would act equally well as antigens. If such were the case, the production of high value toxin and toxoid would be unnecessary; the organisms could be grown in large quantities of glucose broth, spun out in a Sharples centrifuge, resuspended in a small amount of saline, boiled and injected. Such a procedure could easily be carried through by the average laboratory assistant. There are two disadvantages. About 5-10 times more medium would be required than by the usual method, but, on the assumption that consistent results would be obtained, this would be offset by the saving in time and labour accorded to a senior worker, and the saving of laboratory animals. In the immunization of the goats, swelling and oedema of the muscles of the jugular vein region were common. This could doubtless be overcome by flushing the needle out with saline, prior to its withdrawal.

For the immunization of sheep, the "boiled bacilli" method has definite possibilities. In the guinea-pigs, immunized in experiment 4, swelling and necrosis of the injected region (s.c. on abdomen) was the rule. Such reactions, in sheep, would not be tolerated by sheep farmers. However, in view of the high degree of immunity produced by *one* injection, an investigation of methods, capable of preventing the reaction, would be well repaid.

From the purely academic side, the problem is fascinating. A suspension of killed organisms, containing no demonstrable toxin or toxoid, is capable, on injection into animals, of stimulating the formation of antitoxin. It is probable, in the light of the recent work on the immunizing power of specific soluble substances and

polysaccharides when adsorbed on protein or colloids, that the antigen in the boiled suspensions is in the nature of a haptene, linked to, or adsorbed on, the protein of the bacillus.

The experiment, conducted with a bacillary suspension, heated at 60° C., indicates that, in addition to the known exo-toxin, Type B produces an endo-toxin, serologically distinct from the exo-toxin.

It is proposed to continue this investigation, to discover, *inter alia*, the best method of hyper-immunizing animals with boiled bacillary suspensions, a way of overcoming the reactions, produced by them and the physical and serological properties of the endo-toxin.

### CONCLUSIONS.

1. The total antitoxin binding value of a toxoid of *Cl. welchii*, Type B (the "lamb dysentery bacillus") has a direct bearing on its immunizing capacity. The more antitoxin a toxoid binds, the greater is the degree of immunity produced by it.

2. Atoxic toxoid, rendered slightly toxic by the addition of the toxins of *Cl. welchii*, Types A or B proved superior as an antigen to the toxoid, itself. The addition of the toxin of *Cl. septicum* did not increase the immunizing value of toxoids.

3. The intravenous injection of 4-6 sub-lethal doses of Type B toxin, within 4-5 hours may so immunize a guinea-pig that it resists a fatal dose of toxin 24 hours later.

4. Alum-precipitated toxoids proved, on the whole, inferior as antigens to the toxoids, themselves. The addition of agar, colloidal iron and saponin to toxoids did not increase their immunizing power.

5. The washed zinc chloride precipitates of toxoids proved superior as antigens to the toxoids, themselves. The solubility of the precipitate appears to have a direct bearing on the degree of immunity produced.

6. Washed living or washed boiled suspensions of Type B bacilli (free from demonstrable toxin) stimulated the formation of antitoxin when injected into goats and guinea-pigs.

7. Washed suspensions of Type B bacilli, heated at 60° C. for half-an-hour, contain a toxic material, not neutralizable by antitoxin.

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ACKNOWLEDGMENT.

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## Section II.

# Parasitology.

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## The Chemotherapy of Oesophagostomiasis in Sheep—II.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research  
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THE experiments recorded in this paper include only tests which were based on administration of the drugs into the abomasum, i.e. in which the drugs were administered after a small quantity of copper sulphate-solution—see paper by Mönnig and Quin in this issue. The work ran somewhat parallel to the investigations recorded in the latter paper and it will be seen that, as more knowledge was gained in regard to stimulation of the oesophageal reflex, the chemotherapeutic investigations profited by this knowledge.

Quite a number of the tests recorded below were carried out on farms in the vicinity of Pretoria, on account of the fact that it is as a rule difficult to produce artificial infestations with *Oesophagostomum columbianum* in a sufficiently large number of sheep and also because it was considered desirable to work under ordinary farming conditions. These circumstances did not always allow the tests to be carried out as exactly and the results to be followed up as far as one would wish, because the work entailed much transport and loss of time and in several cases the sheep were either sold or moved to another locality soon after the treatment. It was, however, usually endeavoured to attach faeces bags to the treated animals, to replace these daily for several days by fresh bags for the purpose of counting the worms passed after treatment, and to obtain faeces again about a fortnight after treatment for the purpose of making cultures from which the final results could be estimated. The original infestation of the sheep was estimated on the basis of reports made by the owners, post-mortems of one or more cases where possible and the clinical picture presented by the sheep selected out of the flocks for treatment. Although this method is not quite satisfactory, it was known that serious losses due to oesophagostomiasis were occurring on the farms selected and no other worms except *Haemonchus contortus* for which the sheep had as a rule been treated, play an important part in this area.

I wish to record my thanks to Mr. B. J. van der Vyver, Government Veterinary Officer of the Pretoria District, for his assistance in selecting the flocks on which these tests were carried out.

In the course of previous investigations (Mönnig, 1933), it had been found that certain relatively insoluble drugs appeared to produce the best results and such drugs were therefore now again tested in the first instance, the selection of the drugs being influenced to some extent by the previous results.

*Test No. 1.*

Out of a large flock of yearling merino lambs 30 moderately poor cases were selected and marked in six groups of five each. They were allowed to graze—on young succulent grass—and drank water immediately before treatment. Dosed 4 p.m. and the faeces bags removed 24 and 48 hours later. In each case the drug was administered immediately after 10 c.c. 1 per cent. copper sulphate solution.

*Group 1*—each 1 gm. *copper tartrate*. Passed 451 *Oes. col.*\* in 48 hours.

*Group 2*—each 0.95 gm. *arsenious sulphide*. One lamb passed 2 *Oes. col.*

*Group 3*—each 0.85 gm. *copper arsenate*. Passed 604 *Oes. col.*

*Group 4*—each 2.4 gm. *hexachlorethane*. No worms passed.

*Group 5*—each 0.6 gm. *barium arsenate*. Passed 94 *Oes. col.*

*Group 6*—each 0.4 gm. *mercurous sulphate*. Passed 11 *Oes. col.*

It is probable that in some cases worms were passed after the 48 hours. In groups 1 and 3 each of the lambs passed a number of worms each day but, as the lambs were not individually marked, individual records cannot be given. Some six weeks later the owner reported that the lambs of groups 1 and 3 had markedly improved but not the others. None of the lambs had appeared to suffer as a result of the treatment.

*Test No. 2.*

Sheep 1-2 years old, very poor and dull, grazing on green grass but had no water on the day of treatment. Stimulated with 10 c.c. 1 per cent. copper sulphate solution.

*Group 1*—8 sheep, each 0.5 gm. *copper arsenate*. Passed 48 *Oes. col.* in 3 days.

*Group 2*—8 sheep, each twice 0.5 gm. *copper arsenate* on two successive days. Passed 188 *Oes. col.* in 3 days from the first treatment.

*Group 3*—4 sheep, each 5 c.c. *tetrachlorethylene* in 10 c.c. liquid paraffin. Passed 93 *Oes. col.* in 3 days

Faeces cultures made a fortnight later showed that the sheep of group 2 were free of nodular worms, while those of the other two groups were still infested. Again the sheep had not been individually numbered, hence it cannot be stated whether all or only some in each group passed worms.

In the light of knowledge which was gained later it appears probable that the copper sulphate solution was too weak to produce a satisfactory stimulus in some cases and also that the single dose of 0.5 gm. copper arsenate was insufficient. The test had been carried out mainly to ascertain whether such a dose would be sufficient.

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\* *Oes. col.* = *Oesophagostomum columbianum*.

*Test No. 3.*

At Onderstepoort three poor, weak, adult sheep were dosed with 0·85 gm. *copper arsenate* each after 10 c.c. 1 per cent. copper sulphate. The following numbers of *Oes. col.* were passed:—

	1st day after treatment.	2nd day.	3rd day.	4th day.	5th day.	Total.
Merino 41482.....	7	5	48	30	0	90
Merino 41483.....	21	7	38	18	0	84
Persian 41466.....	0	1	0	0	0	1

Subsequent examination showed that the two merinos were clean but the persian still infested. The latter had probably swallowed the drug into the rumen.

*Test No. 4.*

Merinos about 2-3 years old, in poor condition, grazing on green grass but had no water on day of treatment. These sheep were at the time not badly infested as they had been given enema treatments on several occasions. Stimulated with 10 c.c. 1 per cent. copper sulphate.

*Group 1*—5 sheep, each 0·85 gm. *copper arsenate*. Three passed no worms, one 9 and one 190 *Oes. col.* in three days.

*Group 2*—4 sheep, each 1 gm. *copper tartrate*. Passed 11, 24, 30 and 117 *Oes. col.* respectively in 3 days.

*Group 3*—5 sheep, each 1 gm. *cryolite* (mixture of fluorides). Passed 11, 3, 2, 3, 3 *Oes. col.* respectively in 3 days.

*Group 4*—5 sheep, each 5 c.c. *tetrachlorethylene* in 15 c.c. skimmed milk. Passed 0, 0, 1, 1, 3 *Oes. col.* respectively in 3 days.

The sheep were not re-examined later.

*Test No. 5.*

Merinos, 2-tooth, moderate to poor condition but well grown. Fed on dry hay and not kept from water. Stimulated with 10 c.c. 1 per cent. copper sulphate.

*Group 1*—2 sheep, each 5 c.c. *tetrachlorethylene* in 15 c.c. liquid paraffin. Only one passed one *Oes. col.* Later both still infested.

*Group 2*—2 sheep, each 0·42 gm. *mercurochrome*. Only one passed two *Oes. col.* Later both infested.

*Group 3*—2 sheep, each 1 gm. of a relatively insoluble proprietary drug stated to possess anthelmintic properties. No worms passed.

*Test No. 6.*

Merinos and crossbred sheep in poor condition, 1-2 years old. Running on green pasture, not kept from water. Stimulated with 10 c.c. 2 per cent. copper sulphate.

*Group 1*—5 sheep, each twice 0.95 gm. *arsenious sulphide* (on two successive days). Passed no worms. Later still infested.

*Group 2*—5 sheep, each twice 1.1 gm. *copper carbonate*. Only one passed one *Oes. col.* Later still infested.

*Group 3*—5 sheep, each twice 1.75 gm. *bismuth subnitrate*. Passed no worms. Later still infested.

*Group 4*—5 sheep, each 1 gm. *lead tartrate*. Passed 23, 1, 0, 0, 0 *Oes. col.* respectively. Later still infested.

*Group 5*—5 sheep, each 1 gm. *lead arsenate*. Passed 5, 9, 10, 15, 0 *Oes. col.* respectively. Later still infested.

*Group 6*—5 sheep, each 1 gm. *bismuth subgallate* ("Derma-tol"). Passed no worms. Later still infested.

In the above tests copper arsenate and copper tartrate were the two outstanding drugs which gave the most satisfactory results and further tests with them were subsequently made, as will be reported below.

TESTS WITH TETRACHLORETHYLENE.

In other anthelmintic work indications had been obtained to the effect that tetrachlorethylene may sometimes be fairly effective against the nodular worm and further tests with this drug were made at Onderstepoort in order to obtain more information.

Tests with this drug on 11 sheep have been mentioned above and the results were not promising. In some cases the 5 c.c. doses were quite large enough considering the size and condition of the sheep, in other cases they could have been larger. Also, it is possible that the copper sulphate solution used was too weak.

In the further tests 5 per cent. copper sulphate was used as stimulant and the doses of tetrachlorethylene was usually 5 c.c. for lambs of 6-12 months and 10 c.c. for sheep over 12 months. The drug was mixed with mineral oils of various specific gravities and viscosities and other vehicles and adjuvants were tested.

Since this work is still in progress it will be reported upon in a later article, but at this stage it may be mentioned that, although it may be possible to arrive at an effective formula, a simple combination of tetrachlorethylene and a mineral oil does not appear to be satisfactory. The doses required to clean sheep of nodular worms after several treatments are on the border of the limit of safety and further study of the conditions under which tetrachlorethylene is toxic to sheep is urgently required. Moreover, the cost of repeated doses as required for a complete cure are relatively high and would be prohibitive under certain conditions.

## FURTHER TESTS WITH COPPER ARSENATE AND COPPER TARTRATE.

*Test No. 7.*

A flock of full-grown merinos in poor condition and rather dull, grazing on slightly dry pasture, kept from water for 24 hours before and 18 hours after treatment.

*Group 1*—70 sheep, stimulated with 10 c.c. 1 per cent. copper sulphate, dosed each 0.6 gm. *copper arsenate*. Five of the poorest sheep of this group passed 73, 29, 29, 0, 0 *Oes. col.* in 48 hours. One very poor sheep in this group (not of the last five used for worm counts) killed three days after treatment had about 200 *Oes. col.* still alive.

*Group 2*—Of 5 poor sheep, stimulated with 10 c.c. 2 per cent. copper sulphate and dosed each 0.6 gm. *copper arsenate* only one passed 13 and another 1 *Oes. col.* in 48 hours.

*Group 3*—90 sheep, stimulated with 10 c.c. 1 per cent. copper sulphate, dosed each 1 gm. *copper tartrate*. Five of the poorest were selected for worm counts; only one passed 10 *Oes. col.*

*Group 4*—Of 5 poor sheep, stimulated with 10 c.c. 2 per cent. copper sulphate and dosed each 1 gm. *copper tartrate* only one passed 1 *Oes. col.*

The doses of the drugs used were apparently too small for these full-grown sheep, and, according to later experiences, the stimulant was also too weak.

## TOXICITY TESTS.

Since nothing was known with regard to the toxicity of these two drugs it was considered advisable first to gain some knowledge on this point.

*Test No. 8.*

A mixed lot of persian and cross-bred sheep ranging in age from about 5 months to 2 years, all except some of the older ones in moderate or poor condition. Grazing was green and the sheep were allowed to drink immediately before treatment. Stimulant 10 c.c. 2 per cent. copper sulphate.

*Group 1*—40 sheep of various ages dosed each 1 gm. copper arsenate. Two days after treatment a lamb of 4-5 months died with lesions of arsenical poisoning and another of the same age was very ill but recovered.

*Group 2*—40 sheep of various ages dosed each 1 gm. copper tartrate. These sheep remained quite normal.

The owner later reported that these sheep had improved appreciably in condition after treatment.



*Test No. 9.*

Merinos in moderate to poor condition but well grown, age 2-tooth.

Feeding on dry hay, not kept from water. Stimulant 10 c.c. 1 per cent. copper sulphate.

*Group 1*—7 sheep, each twice (on two successive days) 1.25 gm. *copper arsenate*. Four died of arsenical poisoning.

*Group 2*—2 sheep, each twice 2 gm. *copper arsenate*. Both died of arsenical poisoning.

*Group 3*—4 sheep, each one dose 4 gm. *copper arsenate*. Two died of arsenical poisoning.

*Group 4*—5 sheep, each twice 1.65 gm. *copper tartrate*. No adverse effects.

*Group 5*—6 sheep, each twice 2 gm. *copper tartrate*. One died of copper poisoning.

*Group 6*—2 sheep, each twice 2.5 gm. *copper tartrate*. One died of copper poisoning.

*Group 7*—3 sheep, each one dose 4 gm. *copper tartrate*. No adverse effects.

*Group 8*—2 sheep, each one dose 5 gm. *copper tartrate*. No adverse effects.

*Group 9*—6 sheep, each one dose 6 gm. *copper tartrate*. 3 Died of copper poisoning, two others showed symptoms—marked icterus and methaemoglobinuria—but recovered.

Most of these sheep had been infested with nodular worms before treatment but were clean thereafter.

According to the results of the above tests it would appear that a double dose of 1 gm. *copper arsenate* or a double dose of 1.5 gm. *copper tartrate* is within the limits of safety for sheep over 18 months old. Since an overdose of the former leads to arsenical poisoning and an overdose of the latter to copper poisoning, a combination of the two drugs, which would allow cutting down the quantity of each, was now considered, particularly because it was though possible that the two drugs acted on the parasites in different ways.

*Test No. 10.*

110 Cross-bred sheep, various ages from about 5 months to 2 years. Grazing on green pasture and allowed to drink immediately before treatment. Stimulated with 10 c.c. 2 per cent. copper sulphate. Dosed with *copper arsenate* 1 part and *copper tartrate* 2 parts, giving on each of two successive days to lambs up to six months 0.72 gm. (0.24+0.48 gm.), 6-18 months 1.1 gm. (0.37+0.73 gm.) and sheep over 18 months 1.45 gm. (0.48+0.97 gm.). The owner reported later that four days after the second dose a small, weak lamb had died but the other sheep showed no ill effects and improved much after the treatment.

## TESTS WITH MIXTURES.

*Test No. 11.*

*Group 1*—5 cross-bred sheep in fairly poor condition, 1-2 years old, grazing on green pasture and not kept from water. Stimulated with 10 c.c. 2 per cent. copper sulphate. Dosed on each of two successive days with 1.1 gm. of mixture as used in test No. 10. Passed 77, 37, 18, 0, 0 *Oes. col.*

*Group 2*—4 cross-bred sheep similar to group 1 and similar treatment but dosed twice with 1.25 gm. of a mixture copper arsenate: copper tartrate=2:3 (i.e. 0.5 gm.+ 0.75 gm.). Passed 139, 99, 32, 1 *Oes. col.*

In both groups no ill effects were noticed and faeces cultures a fortnight later showed that the sheep were all free of nodular worms.

*Group 3*—About 100 cross-bred sheep of the same flock, treated like the other two groups but dosed twice with 1.4 gm. of a mixture of copper arsenate: copper tartrate=2:5 (i.e. 0.4 gm.+1.0 gm. No faeces were collected but the owner reported later that the flock had completely recovered.

In this connection it should be stated that, when faeces were collected from groups 1 and 2 for cultures a fortnight after treatment the whole flock was treated for infestation with *Oestrus ovis* which appeared to affect a number of the sheep, and which may have accounted for the poor condition, because the majority of the sheep had relatively few nodular worms and some none at all, at the time of treatment. In several instances it has been noticed that the combination of nodular worm and *Oestrus ovis* is very severe on sheep. The nasal maggots produce an irritation which keeps the sheep from feeding properly and an insufficiency of food has very serious effects on sheep infested with nodular worms.

*Test No. 12.*

A flock of 160 full-mouth merinos in very poor condition and rather dull. Grazing on pasture which, though still green, was becoming coarse. Kept from water from 24 hours before first dose until 12 hours after second dose. Stimulant 10 c.c. 2 per cent. copper sulphate. Dosed each twice with 0.5 gm. copper arsenate and 1.25 gm. copper tartrate (i.e. 2:5). Twenty-four of the poorest sheep were selected for collection of faeces and all except four passed nodular worms. They had not been individually numbered, but the total of worms passed was 469, giving an average of 23.5 for the 20 sheep.

A week after treatment the three poorest sheep of the flock were killed; they still harboured roughly 50, 150 and 200 nodular worms.

The day after the second dose two sheep died and the next day another one, apparently of arsenical poisoning. The whole flock appeared to be affected by the treatment and did not improve much later on.

This result led to further toxicity tests:—

*Test No. 13.*

Sheep at Onderstepoort, full grown, 4-6 tooth, in medium to poor condition. Fed dry hay and crushed maize. Stimulant 10 c.c. 2 per cent. copper sulphate.

*Group 1*—50 sheep. Not kept from water but did not drink within a few hours of treatment. Dosed twice with 0.8 gm. copper arsenate and 1.2 gm. copper tartrate. Two sheep died of arsenical poisoning after the first dose. Another five died of arsenical poisoning after two doses.

*Group 2*—20 sheep, kept from water on the days of treatment, dosed at 2 p.m. Each received twice 0.6 gm. copper arsenate and 1.5 gm. copper tartrate. Not affected by treatment, no deaths.

*Group 3*—18 sheep, treated like group 2 but dosed each twice 0.6 gm. copper arsenate and 1.2 gm. copper tartrate. One sheep died a day after the second dose but showed no lesions and the liver contained only traces of arsenic.

*Group 4*—2 lambs about 4 months old, treated like group 2 but dosed each twice 0.4 gm. copper arsenate and 0.8 gm. copper tartrate. No ill effects.

*Group 5*—20 sheep, kept from water from 24 hours before first treatment until 6 hours after second treatment. Dosed each twice with 0.6 gm. copper arsenate and 1.5 gm. copper tartrate. One sheep died of arsenical poisoning a day after the second dose and another four days later. The other sheep did not appear to be badly affected but some were rather dull for a few days.

*Group 6*—20 sheep, treated like group 5 but dosed each twice with 0.7 gm. copper arsenate and 1.75 gm. copper tartrate. Two sheep died after the first dose; one, very poor and weak, died 8 hours after treatment and had all of the remedy still in the abomasum and there was a slight hyperaemia of the mucosa. The other sheep, in fair condition, died 24 hours after treatment, having been prostrated for several hours, and very little of the remedy had yet left the abomasum. There was an acute abomasitis and the liver showed a sufficient quantity of arsenic (0.6 mg.  $\text{As}_2\text{O}_3$  per 100 gm.) to confirm the diagnosis of arsenical poisoning. Three more sheep died of arsenical poisoning two days after the second dose. The rest all appeared to be slightly affected for a few days.

*Group 7*—20 sheep, treated like group 5 but dosed each twice with 0.8 gm. copper arsenate and 2 gm. copper tartrate. Three sheep died of arsenical poisoning, one, three and six days respectively after the second dose. The others also were slightly affected for a few days.

In considering the results of tests Nos. 12 and 13 two points have to be taken separately.

Firstly the question whether the drugs were swallowed into the abomasum in all cases. From information which was obtained at a later stage and which is discussed in another paper (see article by Mönnig and Quin in this issue) it appears very probable that this was not the case. It was later found that full-grown sheep, and particularly if they are in poor condition, are not well stimulated by 2 per cent. copper sulphate. In addition there is the factor of fluidity of the ruminal contents—if the contents are fairly fluid the drug passes to the abomasum more frequently than when the contents are on the dry side, and the latter was probably the case with many of these sheep, especially groups 2-7 which were receiving dry food and were kept from water. If the drug falls into the rumen it is practically lost as far as the nodular worm is concerned, because the small quantities which would pass through over a prolonged period would probably not reach the worms, since the drug would become dissolved in the abomasum and be absorbed. Since the absorption would be slow and spread over a considerable period of time, the danger to the sheep would be relatively small. In test No. 12 probably one or both of the two doses were in many cases swallowed into the rumen, hence the low efficacy. The fact that the three poorest sheep were eventually killed for examination is also significant because these were the worst cases for stimulation and would therefore show the least successful result.

Secondly there is the question what happens when the remedy is swallowed into the abomasum. Both the drugs are relatively insoluble in water but more soluble in an acid medium. The following degrees of solubility were determined by Mr. P. M. Bekker of the chemistry section. Solubility at  $\pm 42^{\circ}\text{C}$ .:—

	In 0.25% HCl.	In 1% HCl.
Copper arsenate.....	0.44%	1.80%
Copper tartrate.....	0.64%	1.44%

Information on the acidity of the abomasal contents of sheep appears to be scanty but it seems that the degree of acidity may vary from about neutral to 0.1 per cent. or even more (HCl) and would be highest in the case of sheep in active process of digestion. It also seems reasonable to conclude that when concentrates are fed and water is withheld there would be a high degree of acidity.

Further, the length of time the drugs remain in the abomasum would be important, since a long stay would afford greater opportunity for the drugs to become dissolved. In group 6 the drugs remained in the abomasum for 8 hours in one case and about 24 hours in another. Although these periods are abnormally long, a similar state of affairs may have obtained in some of the other sheep.

One may therefore conclude that, if the degree of acidity in the abomasum is high the drugs will become dissolved to a fair extent. The dissolved drugs would then be readily absorbed and may lead to poisoning of the sheep and furthermore they could not be effective against the nodular worms in the colon. The longer the drugs remain

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in the abomasum the greater becomes this risk. Unfortunately we have no definite knowledge to-day in regard to the physiology of the abomasum and the factors which control the passage of ingesta through the pylorus on which one could base attempts to accelerate the passage of drugs through the abomasum. While starvation is obviously contraindicated, it would seem that the most favourable condition to be aimed at under the circumstances is that of normal, active digestion in which the ingesta pass through the abomasum and intestine at a reasonable speed. Water should not be withheld and fresh, green grass appears to have a favourable influence.

If one has to deal with a reasonably short stay of the drugs in the abomasum, it may be possible to neutralise the acid for that period and this was therefore the next step contemplated. Both the drugs are relatively soluble in an alkaline medium, as can be readily shown in a test-tube. Since the amount of acid in the abomasum varies within wide limits, a neutralising agent given in sufficient quantity to cope with a high degree of acidity would often be in excess if acidity is low and should then not produce a sufficient degree of alkalinity to dissolve the drugs. The neutralising agent has therefore to be an alkaline substance which does not ionise to any marked extent. These requirements appear to be satisfactorily fulfilled by slaked lime (calcium hydroxide). Tests in the laboratory showed that when the drugs were added to dilute hydrochloric acid, the addition to the drugs of slaked lime in excess of the acid produced an almost neutral medium with no appreciable solution of the drugs, while sodium bicarbonate instead of slaked lime produced an alkaline medium and a fair proportion of the drugs became dissolved. Subsequent tests on sheep also showed that sodium bicarbonate did not improve matters while slaked lime brought about a definite improvement.

## Test No. 14.

The following mixtures were tested:—

	Dose* for sheep—		
	3-6 months.	Over 6 to 18 months.	Over 18 months.
<i>Mixture I.</i>	Gramme.	Gramme.	Gramme.
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.05	1.4	1.75
<i>Mixture II.</i>	Gramme.	Gramme.	Gramme.
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Sodium bicarbonate, 3 parts.....	0.45	0.6	0.75
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.5	2.0	2.5
<i>Mixture III.</i>	Gramme.	Gramme.	Gramme.
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Calcium hydroxide, 3 parts.....	0.45	0.6	0.75
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.5	2.0	2.5

\* Single dose, to be repeated the following day.

A flock of 523 sheep running on green grass, all well over 18 months old, was treated as follows:—

The poorest sheep were selected, some being in a very bad condition. This lot of 146 was divided into four groups:—

*Group 1*—25 sheep, 24 hours without water, drank immediately before treatment. Mixture I.

*Group 2*—48 sheep, 24 hours without water, drank immediately before treatment. Mixture III.

*Group 3*—25 sheep, 24 hours without water, drank immediately after treatment. Mixture I.

*Group 4*—48 sheep, 24 hours without water, drank immediately after treatment. Mixture III.

The rest of the flock, in moderate to good condition, was allowed to graze and drink and some drank immediately before treatment.

*Group 5*—50 sheep, mixture I.

*Group 6*—100 sheep, mixture II.

*Group 7*—227 sheep, mixture III.

All the sheep were stimulated with 10 c.c. 2 per cent. copper sulphate and given a double dose, i.e. on two successive days.

Groups 1-4 were kept in a suitable enclosure overnight after both treatments and the droppings showed that numerous nodular worms had been passed. The shepherd also reported that worms were passed during the daytime up to three days after the second treatment. Groups 5-7 also showed nodular worms in many lots of faeces found in their pasture. The sheep of groups 2, 4 and 7 which had received the mixture containing slaked lime showed no ill effects and definitely looked more lively than many of the other sheep did shortly after treatment.

A day after the second dose one sheep each of groups 1, 3 and 6 died of arsenical poisoning and during the following two days another two of group 6 died, thus showing apparently that the sodium bicarbonate was not satisfactory.

From the number of worms passed by groups 1-4 it was obvious that a considerable proportion of the infestation had been removed from the flock, but whether the treatment had been effective in all cases, especially the very poor sheep, remained to be seen. The five poorest sheep were therefore selected from each of these four groups

## CHEMOTHERAPY OF OESOPHAGOSTOMIASIS IN SHEEP.

(1-4) and slaughtered. They harboured the following numbers of nodular worms (given in round numbers in the positive cases with a fair or heavy infestation):—

Group 1—0, 0, 11, 50, 200.

Group 2—0, 0, 80, 150, 200.

Group 3—5, 20, 26, 120, 120.

Group 4—0, 80, 100, 200, 200.

This result would seem to indicate that, as has been mentioned before and was later confirmed, sheep in very poor condition are bad subjects for stimulation of the oesophageal reflex and that the efficiency of treatment depends not only on the drugs used but to an equal degree on the route of passage through the stomach.

Another flock of 300 yearling lambs in moderately poor condition were treated, again using 10 c.c. 2 per cent. copper sulphate as stimulant and giving a double dose of mixture III. These lambs grazed on green grass and were allowed water freely. Many of them drank shortly after being given the first dose and also shortly before the second dose. During the night after the first dose many worms were passed. After having received the second dose the sheep were driven slowly to another farm and the owner reported later that no ill effects had been observed and the lambs had improved markedly.

### *Test No. 15.*

A toxicity test was now made with mixture III as used in test 14. The sheep were in good condition and were fed dry hay and crushed maize. They were kept from water for 24 hours before each treatment with the intention of getting them to drink immediately before dosing, but the weather was cold and on both days only a few of the sheep drank a little. Again 10 c.c. 2 per cent. copper sulphate was used as stimulant.

*Group 1*—7 sheep, each twice 3 gm. mixture III (copper arsenate 0·6, lime 0·9, copper tartrate 1·5 gm.).

*Group 2*—7 sheep, each twice 4 gm. mixture III (0·8, 1·2, 2·0 gm.).

*Group 3*—6 sheep each twice 5 gm. mixture III (1·0, 1·5, 2·5 gm.).

No ill effects were observed in any of these sheep.

The day after the second dose one sheep of each group was selected which had swallowed one or both doses into the abomasum, as far as this could be ascertained from the appearance of the faeces. These three sheep were killed and carefully examined with the following results:—

*Group 1*—normal except for a very slight hyperaemia of the duodenum, which would be of practically no consequence.

Liver shows a trace of arsenic.

*Group 2*—normal. Liver shows a trace of arsenic.

*Group 3*—a very slight hyperaemia of the abomasal mucosa and the anterior 15 cm. of the duodenum; of no practical importance. Liver shows a trace of arsenic.

*Test No. 16.*

A critical test with mixture III, giving the same doses as in test 14, was now made on some very poor sheep, using 10 c.c. 5 per cent. copper sulphate as stimulant. In the experiments on deglutition it had meanwhile been found that a stronger bluestone solution gave a better stimulus in such poor sheep. Some of the sheep died of poverty shortly after treatment while the others were killed in deglutition tests and in every case the intestines were examined for the presence of nodular worms.

Age of sheep.	Nodular worms passed.	Worms remaining.	Approximate efficacy.
4-tooth.....	6	about 30	0
	171	30	0·5
	82	0	1
6-tooth.....	212	0	1
9 months.....	48	about 20	0·5
	177	0	1
	1	0	1
	2	2	0·5
	10	0	1
	26	0	1
	157	0	1
	892	82	8·5/11 = 77%

This result was not yet quite satisfactory, especially with regard to the older sheep. Some may have swallowed only one of the two doses into the abomasum. It was later found that 10 per cent. copper sulphate produced better results in deglutition tests. Further tests were made on farms with 5 per cent. copper sulphate as stimulant and using the same doses of mixture III as in test 14. In all cases the results were satisfactory, i.e. many worms were passed and the sheep improved in condition.

In one case the first opportunity arrived of treating young lambs of 3-4 months. They were in very poor condition, weak and rather small for their age. The lambs were separated from the ewes for four hours before up to three hours after treatment. A double dose of mixture III was given (each dose copper arsenate 0·3 gm., lime 0·45 gm., copper tartrate 0·75 gm.). Of 80 lambs so treated 30 died within a fortnight. Unfortunately the owner made no observations with regard to the cause of death and only reported the deaths a few weeks later. However, the proportion of deaths was higher than in the untreated controls. The owner then treated another 50 lambs with a single dose. These passed many worms and one death occurred about 10 days after treatment, probably on account of weakness.



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Since there appeared to be a certain degree of danger in using the doses previously arrived at, it was decided to reduce the doses for young sheep and the following was now decided on:—

	Dose for—		
	3-6 months.	Over 6 to 18 months.	Over 18 months.
	Gramme.	Gramme.	Gramme.
Copper arsenate.....2	0.2	0.36	0.5
Calcium hydroxide.....3	0.3	0.54	0.75
Copper tartrate.....5	0.5	0.9	1.25
Dose No.....	1 : 1.0	2 : 1.8	3 : 2.5

Sheep in very poor condition should receive the next smaller dose on the second day, very poor lambs only one dose.

According to these measures about 2,000 sheep of various ages, including young lambs, were treated on seven farms in different parts of the Union. The treatment was carried out by farmers with the assistance of Government Veterinary Officers. For stimulation 5 per cent. copper sulphate was used. No deaths occurred as the result of treatment. In some cases faeces were collected and the droppings of other sheep were examined where the animals had slept after treatment. The impression was obtained that a large proportion of the infestation was eliminated. Incidentally it was noted that many *Haemonchus contortus* and *Moniezia* were also passed and heads of the tapeworms were recovered from the faeces in some cases.

Meanwhile it had been determined that a 10 per cent. copper sulphate solution produced satisfactory stimulation even in very poor conditioned, full-grown sheep and that 2.5 c.c. was sufficient for this purpose. It was therefore decided to test the efficacy of the treatment again with the stronger stimulant.

## Test No. 17.

Ten 2-tooth sheep in very poor condition and rather dull, feeding on dry hay and crushed maize, not removed from water. Stimulant 2.5 c.c. 10 per cent. copper sulphate. First day dose No. 2, second day dose No. 1.

According to faeces cultures made seven days before treatment all these sheep had nodular worms, though not severe infestations. Two had *Haemonchus contortus*. They passed respectively 16, 9, 52, 3, 17, 16, 20, 32, 23 and 203 (total 391) nodular worms. Three died six to fourteen days after treatment of poverty and had no worms left. Faeces cultures of the other sheep showed no residual infestation with nodular worms or *Haemonchus* except one culture in which a single oesophagostome larva was found. Some of these sheep were later again examined and treated but appeared to be free of nodular worms.

Unfortunately no older sheep in poor condition with nodular worm infestation were available for these tests. Such sheep are important as reservoirs of infection and therefore require to be cleaned. As a rule, however, it is the young sheep up to 2-tooth that suffer and for which a remedy is mainly required. Judging from the results obtained in deglutition tests on old, poor conditioned sheep, it is very probable that two or three treatments would clean such cases, while many would be cleaned already by one treatment.

#### *Test No. 18.*

Although test No. 17 had shown that one treatment may suffice for relatively young sheep, repeated treatments would probably be given in practice on farms and it was therefore considered desirable to test the safety of repeated treatments, at short intervals.

A month after test No. 17 two of the sheep used in that experiment and another four of which one was infested, were treated giving dose No. 2 each time. All these sheep were in very poor condition, the state which is reached by some sheep with nodular worm infestation when, even if the parasites are removed, the animals hang on and either die after weeks or recover very gradually. The one infested sheep passed 31 nodular worms and none of the animals showed any adverse effects.

A week later the treatment was repeated, again giving full doses. No worms were passed and no adverse effects were noted.

Another week later the same treatment was repeated and two new infested sheep, also in poor condition, were included. These latter two passed all their nodular worms. Again no ill effects were noted.

It appears therefore that treatment can safely be repeated at short intervals, although in practice intervals as short as seven days would rarely be contemplated.

#### DISCUSSION.

If the results are considered together with the work done on stimulation of the oesophageal reflex, which ran parallel, it becomes quite clear that the question of deglutition is an important part of the treatment against oesophagostomiasis and that it will be equally important in the chemotherapy of other gastro-intestinal worm diseases of ruminants.

The mixture of copper arsenate, calcium hydroxide and copper tartrate appears to fulfil the requirements of an effective remedy against nodular worm in sheep. If the remedy does not have the desired effect, the evidence appears to show that deglutition into the abomasum was not satisfactory.

The remedy appears to stimulate secretion of mucus in the colon, so that in many cases the faeces become soft or mixed with a fair amount of mucus and sometimes a sheep may even pass a mass of mucus. In a few cases it has been observed that live nodular worms

were passed with such mucus, as may be expected. The remedy does, however, not act by virtue of this property, since in many of the cases on which careful observations were made the worms were passed in normal or slightly pasty faeces.

It may be necessary to explain why the idea of double dosing was adopted. The reasons will be given in order of their estimated importance:—

1. In the case of a parasite with feeding habits such as those of *Oesophagostomum columbianum* and a drug which probably acts as an internal poison it appears desirable to have a long period during which action of the drug on the parasite is possible.
2. Since the oesophageal reflex cannot yet be stimulated in 100 per cent. of cases, but in well over 50 per cent., and since the efficacy of one of the doses is very probably more than half that of the two doses, some sheep which swallow one dose into the rumen may swallow the other into the abomasum and the total result in a flock will be better than if a single large dose is given.
3. The total quantity of the drugs administered can be greater and each dose smaller than one single dose.

It is not claimed that a completely satisfactory remedy has been found for oesophagostomiasis in sheep. Further tests on a large scale will have to show what degree of efficacy can be obtained under different conditions and whether certain yet unknown factors may not introduce an element of danger. Moreover, further possible improvements with respect to stimulation of the oesophageal reflex may improve the results. It is, however, expected that a few treatments at the correct time of the year will stop the disease and lead to satisfactory control of the parasite, particularly if treatment is carried out with a view to removing the source of infection at a suitable season.

The Department of Agriculture has decided to issue to farmers about half a million double doses of this remedy for the purpose of a test. The directions for use issued with the remedy are given below and from these it can be seen in which way it is intended that the remedy should be used in the treatment and control of the disease. It might be added here that the disease is mainly important in areas with a summer rainfall (October to March) and a relatively dry winter.

With regard to costs it may be stated that, if the remedy should prove satisfactory and is later issued on a large scale, it is expected that the costs should not be higher than about 1s. per 100 double adult doses.

I would like to express my thanks to Drs. Malan and Graf and other members of the Chemistry Section for making the copper arsenate and copper tartrate used in these tests and for assistance in various other ways.

## SUMMARY.

1. Chemotherapy for oesophagostomiasis must be based on the administration of the remedy into the abomasum. At first a 1 per cent. copper sulphate solution was used for stimulation of the oesophageal reflex, then higher concentrations until finally satisfactory results were obtained with 2.5 c.c. of a 10 per cent. solution.

2. Preliminary tests were made with 15 different chemicals which had either previously given indications that they may be effective, or appeared, according to their chemical and physical properties, to be suitable. Of these copper arsenate and copper tartrate gave rather outstanding results.

3. Both these drugs were found to be dangerous in doses which would be large enough to effect a cure. An overdose of copper arsenate leads to arsenical poisoning while an overdose of copper tartrate leads to copper poisoning.

4. A mixture of the two drugs produced variable results which were found to be connected with the variable amount of acid in the abomasum. Since the drugs are relatively soluble in acid and alkaline media, high stomach acidity would cause solution and absorption of the drugs with consequent poisoning of the sheep and little or no effect against the parasites. Calcium hydroxide was found to be a suitable corrective and was incorporated in the mixture. Effective doses of this mixture were found to possess a suitable degree of safety, even if treatment is repeated at relatively short intervals.

5. Taking into account all ages and conditions of sheep, the efficacy of one treatment, i.e. a dose on each of two successive days, is expected to reach at least 75 per cent. If only young sheep under 2 years of age are treated the efficacy will be considerably higher.

6. It has incidentally been observed that the mixture has a fair degree of efficacy against *Haemonchus contortus* and *Moniezia expansa*.

7. Measures for treatment and prevention of oesophagostomiasis by means of a suitable remedy under South African conditions are outlined.

## ADDENDUM.

The following information is available with regard to the test referred to above:—

Reports have been received on 94,263 sheep treated by 103 farmers. The total losses registered after treatment are 231 or 0.245 per cent. Most of the sheep were in poor condition and were grazing on dry pasture. In some cases the deaths were definitely due to the treatment; one flock which was affected had been starved; in a few cases the powder was inhaled and in some other cases the deaths were due to weakness and would probably have occurred in any case. In two cases, accounting for losses of 30 and 28 sheep respectively, the sheep were receiving fairly large rations of crushed maize. From

## CHEMOTHERAPY OF OESOPHAGOSTOMIASIS IN SHEEP.

observations made it appears that crushed maize or other grain frequently reaches the abomasum from the fore-stomachs and there is then a tendency towards retention of the abomasal contents until the grain has been softened and broken up. This dietetic factor seems to be of considerable importance in causing stagnation in the abomasum, which is dangerous in connection with this treatment.

A few farmers were not satisfied with the efficacy of the remedy since only a small number of worms were observed to be passed or some sheep killed after treatment still had a residual infection. The large majority, however, reported that large numbers of worms had been passed and they were well pleased with the result. In several cases it was reported that deaths due to oesophagostomiasis stopped immediately and with few exceptions the reports contain the statement that the sheep improved markedly after treatment. It was also noted in several cases that the animals started to feed much better than before from the day after the administration of the second dose.

From the experience gained the following appear to be important points to observe and these are being incorporated in the directions for use of further issues of the remedy.

1. The sheep should get no grain ration or salt lick from two days before until a day after treatment.
2. The sheep should have access to water immediately before being dosed.
3. If the pasture is dry it is very desirable to allow green food for a few hours before each dosing.
4. In order to prevent inhalation the powder should be administered immediately after the bluestone solution, so that it reaches the pharynx when the sheep has closed the glottis in preparation for swallowing the bluestone solution.

## LITERATURE CITED.

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## APPENDIX.

### NODULAR WORM REMEDY: DIRECTIONS FOR USE.

The remedy is issued in tins which contain a sufficient quantity for single dosing of 100 sheep over 18 months, or 140 sheep between 6 and 18 months, or 250 lambs of 3 to 6 months.

## METHOD OF TREATMENT.

The sheep must not be kept from food or water before treatment and may graze again immediately after treatment but should have no water for 1-2 hours. They may also be treated towards evening and then remain in the kraal overnight.

Suckling lambs must not have a drink four hours before and four hours after treatment.

The efficacy of the remedy depends on its being swallowed directly into the fourth stomach, and this is brought about by administering a small quantity of bluestone solution immediately before the remedy. The moment the bluestone reaches the throat of the sheep the large stomach closes and remains closed for 15 seconds.

The treatment therefore consists of two parts as follows:—

1. The spoon marked X is filled with a 10 per cent. bluestone solution—to prepare this dissolve 1 pound bluestone in 1 gallon of water, or 2 ounces to 1 pint water, or 2½ ounces to 1 bottle water—open the sheep's mouth well and pour the bluestone alongside of the tongue so that it runs down into the throat. For small lambs a ½ spoonful is sufficient.
2. The correct measuring spoon is filled with the remedy beforehand and is kept ready to be emptied on to the back of the tongue immediately after giving the bluestone, without having closed the sheep's mouth. Directly after the administration of the powder the mouth is closed and the sheep released.

The sheep have to get a *double dose* of the remedy and are therefore treated in the same way on two successive days.

Before using the remedy it is desirable to empty the contents of each tin into a suitable receptacle and to stir it well.

A quantity of the powder is then placed into the tin with the crossbeam (as used with Government Wireworm Remedy and obtainable from the Laboratory). The correct measuring spoon is filled lightly, without pressing against the side of the tin, and scraped off level against the underside of the crossbeam.

The remedy is administered by means of the measuring spoon. Should the spoon become wet and the powder cling to it, it must be wiped. The following are the correct doses:—

For lambs 3 to 6 months, spoon No. 1.

For lambs over 6 to 18 months, spoon No. 2.

For sheep over 18 months, spoon No. 3.

Sheep which are very weak or small for their age should get a smaller dose on the second day, e.g. a 2-tooth sheep first day spoon No. 3 and second day spoon No. 2. Weak lambs are dosed once only. Lambs under 4 months should not be dosed unless it is really necessary.

## RESULTS OF TREATMENT.

The remedy causes much slime to be formed in the large intestine and the droppings therefore often contain much slime after treatment and have a blue-green colour due to the remedy. It is expected that one treatment (double dosing) will remove at least three-fourths of the worms and many sheep will be clean. It has also been noticed that the remedy is effective against wireworms and tapeworms in sheep.

## WHEN AND HOW OFTEN TO TREAT.

In order to understand this properly, the life-cycle of the nodular worm must be explained. The worm eggs are passed in the droppings of the sheep and hatch in the pasture if it is moist and warm. The small worms are ready to infect sheep after a week and can live in the pasture for several months. In winter when it is dry the pasture becomes clean, because the eggs and young worms are killed by the prolonged drought. Infested sheep are the source of infection for the next summer. (They should therefore be cleaned during the winter.)

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When the young worms are swallowed by a sheep they bore into the wall of the intestine, causing the well-known nodules to develop. Here they may stay for 5 days to 3 months, then they return to the inside of the gut, pass to the large bowel where they grow adult and begin to lay eggs after about 5 weeks. (One treatment therefore does not clean sheep, since the young worms in the nodules are not killed.)

The sheep therefore infect the pasture when the rainy season starts and they themselves become further infected during the summer. When the pasture becomes dry and feeding poor about April, the sheep begin to die of the worms which they harbour. (They should therefore be treated before this time arrives.)

The following is therefore recommended :—

1. Badly infested sheep may be treated at any time of the year to cure them and three treatments with intervals of 4-6 weeks are necessary. (It will, however, do no harm if the sheep are treated a few times at intervals of 2 weeks.)
2. Under ordinary circumstances it will suffice to treat every six weeks from January or February until July. The first few treatments are to cure the sheep before they should die in April-May. The further treatments are to clean them thoroughly during the winter, so as to remove the source of infection for the following year.
3. It is expected that after a few years of treatment as indicated under 2, the nodular worm as a pest will disappear. In order to prevent further trouble it is strongly recommended that sheep should then still be dosed annually three times with intervals of six weeks between the months of April and July.

*Wireworms.*—Sheep may be dosed for wireworms a fortnight before or after dosing for nodular worm. Since the nodular worm remedy kills wireworms fairly effectively, the usual treatment for wireworms can be carried out under scheme 2 outlined above each third week after a treatment for nodular worm. At other times sheep should be dosed for wireworms throughout the year at intervals of 3 weeks, or in very wet summer months every 2 weeks, using the ordinary wireworm remedies.

### SPECIAL MEASURES.

*Ewes.*—It is not advisable to treat ewes from about a month before lambing up to about a fortnight thereafter. Pregnant ewes must be handled carefully.

The sheep should not be chased about unduly during or after dosing and it is desirable to use a suitable crush for dosing.

As the remedy is poisonous it should be handled carefully and sheep should not get more than the doses recommended.

### LASTING PROPERTIES OF THE POWDER.

The powder does not decompose or lose strength, but it is advisable to keep the tins well closed and to store them in a dry place.

### PRICE-LIST.

Tins of powder containing 100 single doses for sheep over 18 months, each 1s. 3d. (for one treatment of 100 adult sheep two such tins are required).

Measuring spoons—set of 4 (X, 1, 2 and 3), 5s.; single spoons, 1s. 3d. Measuring bowl with crossbeam, 6d. each. Post or rail-free to any post of the Union.

These articles are delivered only for cash or c.o.d. by rail or post. Orders to be addressed to the Director of Veterinary Services, P.O. Onderstepoort, Pretoria.

All Laboratory products are carefully tested before issue but are issued solely at buyer's risk. The Government cannot be held responsible for any losses or accidents which might possibly occur after their use.

*N.B.*—Empty tins should not be returned.

## Section III.

# Poisonous Plants.

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## **The Toxicity of Pumpkin Seed (*Cucurbita pepo* L.).**

By DOUW G. STEYN, B.Sc., DR. MED. VET. (VIENNA), D.V.Sc.,  
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THIS investigation was prompted by the fact that recently a report was made by a farmer in the Volksrust district that his sheep, which were running on a land littered with "boer pumpkins", developed symptoms of paralysis and died. The animals were feeding extensively on the pumpkins and were also eating the seed. Stockowners have repeatedly suspected pumpkin seed of causing "craziness" and symptoms of paralysis in stock, ostriches and poultry.

The following experiments were conducted with the seed, which was freshly removed from mature "boer pumpkins":—

(1) A one-year-old sheep (No. 40623, weighing 22 kg.) received 400 grams of the fresh seed per stomach-tube daily on three consecutive days.

*Result.*—On the third day of the experiment the animal showed diarrhoea. It showed no symptoms of illness and was feeding well. On the third day after the last dose the animal appeared to be in normal health again.

(2) Rabbit A (1.9 kg.) received 20 grams of the fresh seed per stomach-tube daily on three consecutive days.

(3) Rabbit B (1.8 kg.) received 40 grams of the fresh seed per stomach-tube daily on three consecutive days.

*Result.*—The animals developed no symptoms of poisoning.

It should be mentioned that the entire fresh seed was minced and drenched with water.

### LITERATURE.

It is well known that pumpkin seed has been and still is being used as a taenicide both by medical practitioners and laymen. For several decades the seed has been recognized as a tapeworm remedy by the United States Pharmacopoeia. Its anthelmintic action is, however, doubted by some investigators. It should, however, be mentioned that others apparently achieved success by treating cases of tapeworm with pumpkin seed. In 1875 Heckel (Power and Salway, 1910) was able to expel tapeworms by means of the membrane surrounding the embryo. He believed the active principle to be a resin.

## TOXICITY OF PUMPKIN SEED.

Wolff (Power and Salway, 1910) found this resin to be an efficient taenifuge in doses of 1.0 gram. Power and Salway (1910) isolated a fatty oil and a resin from fresh pumpkin seeds obtained from the United States. Dr. Dale, Director of the Wellcome Research Laboratories, found that doses of 30 c.c. of the fatty oil, isolated by Power and Salway, had no effect on tapeworms in dogs. Also the resin in doses of 0.9 gram had practically no effect on tapeworms in these animals. In addition to these experiments several medical practitioners administered 15 to 60 c.c. of the oil and 1.0 gram of the resin to human beings suffering from tapeworms without achieving any results. In a later publication by Power (1912) he again refers to the value of pumpkin seed as a taenifuge, and states there are no grounds for the incorporation of the seed in the pharmacopoeia. Rath (1929) mentions the following cases: (a) Slop determined as early as 1881, that 150 to 200 seeds, or 15 to 20 grams of the oil contained in the seed, provide a safe means of expelling tapeworms. He stressed the point that the pumpkins should be grown in a warm climate. (b) In 1885 Hartwich investigated the anthelmintic properties; and (c) in 1915 Stefanowicz reported that he had treated fifty cases of infections with *Taenia saginata* and *Taenia solium* with pumpkin seed, and that in one case only was it necessary to repeat the treatment.

Weiss (1928) states that he was able to remove *Taenia saginata* from himself with pumpkin seed. The head of the tapeworm was also expelled a few hours after he had taken 200 shelled seeds followed by a dose of castor oil. The treatment with pumpkin seed was applied after an unsuccessful attempt to remove a tapeworm with *filmaronic oil*. He also mentions other cases of tapeworm infections which were treated successfully with pumpkin seed. He recommends the seed as a safe and efficient tapeworm remedy in doses for adults of from 150-200 seeds. They must be fresh, and must be taken macerated into a porridge with milk. Castor oil should be administered about an hour after the seeds were taken. It is stated that the ingredient (or ingredients) responsible for the expulsion of tapeworms is situated in the thin greyish layer underneath the shell of the seed. Rath (1929) failed to confirm this contention. The seeds should therefore be carefully shelled so as not to remove this layer.

Rath (1929) conducted some experiments with pumpkin seed upon a small earthworm, *Allolophora foetida*. Rath extracted from the seed an oil, which he found to be very poisonous to the earthworms, an emulsion of 1:500 with water killing earthworms in 3-4 hours. After extraction with ether, petroleum ether, or alcohol, the seed residue was found to be non-poisonous to earthworms.

Wehmer (1929) gives a full account of the chemical composition of the pumpkin and its seed.

The use of "Melon pumpkin seed", the fresh seeds of *Cucurbita maxima* Duchesne, as a taenifuge is mentioned in The British Pharmaceutical Codex, 1934, p. 374. It is stated that its use should be preceded by the administration of a saline purge, and followed by a dose of castor oil.

Immelmann (1933) also reports favourably on the use of pumpkin seed as a taenifuge.

## SUMMARY.

(1) No experimental evidence was obtained to support the contention that pumpkin seed produces paralysis in stock. A young sheep drenched with large amounts of fresh seed developed transient diarrhoea. It is possible that pumpkins grown in different areas differ in their effects on stock.

(2) The evidence found in the literature in regard to the value of the seed as a taenifuge is contradictory. It is possible that the ingredient (or ingredients), which is responsible for the effect of the seed as a taenifuge, varies in the specimens of pumpkin grown in different areas.

(3) The fatty oil and the resin contained in pumpkin seed appears to be non-toxic to animals and human beings when given in moderate doses.

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## The Occurrence of Cyanogenetic Glucosides in South African Species of *Acacia*.

### II. Determination of the Chemical Constitution of Acacipetalin. Its Isolation from *Acacia stolonifera*, Burch.

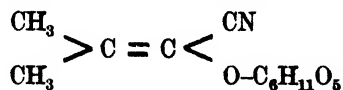
By CLAUDE RIMINGTON, M.A., Ph.D., B.Sc., A.I.C., Research Fellow under the Empire Marketing Board.

STEYN AND RIMINGTON (1935), in the first paper of this series, reported the occurrence in several South African species of *Acacia* of significant quantities of cyanogenetic substances. Among the species investigated, *Acacia lasiopetala* Oliv., and *Acacia stolonifera*, Burch. were found to yield the largest quantities of prussic acid.

The glucoside was isolated from the former species and shown to correspond with none of the known cyanogenetic glucosides. It was proposed to name it *Acacipetalin*.

In the present communication are recorded improvements in the method of preparation and also the isolation of Acacipetalin from *Acacia stolonifera* (see Fig. 1).

The constitution of Acacipetalin has been elucidated. It is the glucose ether of dimethylketenecyanhydrin.



The only reference to the presence of cyanogenetic glucosides in species of *Acacia*, other than those studied by Steyn and the present writer, is contained in the work of Finnemore and Gledhill (1928) and Finnemore and Cox (1930). The latter isolated the glucoside sambunigrin, benzaldehyde-cyanhydrin glucose ether, from the Australian species *Acacia glaucescens* and *Acacia cheelii*. As pointed out by Steyn and Rimington, the South African species of *Acacia* belong, botanically, to a different group in the genus than do the Australian species, and it is therefore of very great interest to find that the glucoside contained in *Acacia lasiopetala* and *Acacia stolonifera* (the only two species so far studied) is in no way related to sambunigrin.

#### OCCURRENCE OF CYANOGENETIC GLUCOSIDES IN S.A. ACACIA.

Accompanying acacipetalin, the substance *Pinit*, inositol monomethyl ether, was found in *Acacia lasiopetala*.



Fig 1—*Acacia Stolonifera*, Burch  $\frac{1}{2}$  natural size

#### IMPROVEMENTS IN THE METHOD OF ISOLATION OF ACACIPETALIN.

It is presumably the presence of Pinit in the *Acacia* species studied which renders the isolation of the cyanogenetic glucoside so difficult. Pinit, or inositol monomethyl ether, has solubilities and physical properties very closely resembling those of Acacipetalin. Thus, both are very soluble in water, sparingly soluble in hot ethyl acetate and insoluble in ether, chloroform or benzene. Whilst the glucoside is sparingly soluble in hot absolute alcohol, however, pinit is practically insoluble in this solvent and it was by means of this difference that their separation was originally achieved before the identity of the non-glucosidal substance was known.

Since pinit can be precipitated by basic lead acetate and ammonia, it was hoped that a more rational separation could be accomplished by the use of these reagents. The surmise was

justified although some glucoside was lost in the lead precipitate. Thus, a residual syrup, from *Acacia stolonifera*, giving strongly positive glucosidal reactions, but from which nothing could be induced to crystallise, was dissolved in water and 1 gm. of cadmium nitrate added, followed by 5 c.c. of saturated basic lead acetate solution and sufficient ammonia to cause complete precipitation. The precipitate was removed and excess of lead precipitated from the filtrate by hydrogen sulphide. The filtered liquid was neutralised, a little solid calcium carbonate added and evaporated to dryness upon the water bath. The residue was exhausted with hot ethyl acetate from which a quantity of glucoside separated in crystalline form as the solution cooled.

Another modification tried out was the process described by Hérissé (1932) as a general one for the extraction of glucosides, successful in many cases where ordinary methods failed.

An extract of *Acacia lasiopetala* was made with boiling 96 per cent. alcohol, the alcohol removed in the presence of a little calcium carbonate by vacuum distillation and the residue taken up in water (volume equal to the weight of plant taken) and filtered. By trial upon an aliquot, diluted, the quantity was determined of basic lead acetate solution necessary for complete precipitation. This amount was then added to the main bulk plus about 10 per cent. in excess. Anhydrous sodium sulphate was then stirred in, using 1 gm. to every 0.75 c.c. of basic lead acetate solution (or 1 gm. to each 1 c.c. of original extract) followed by calcium carbonate in the proportion 0.25 to 0.5 gm. for each 1 gm. of sodium sulphate. The mixture was well stirred at intervals and when sufficiently solid was spread out on a large tray to dry. Dehydration was completed in the vacuum desiccator. The powdered mass was then introduced into a Soxhlet thimble and continuously extracted with boiling ethyl acetate. From the extract, some glucoside crystallised on cooling and a further quantity was obtained by evaporating to dryness, redissolving the deeply coloured residue in water, decolorising by charcoal, evaporating and again extracting the dry residue with successive portions of boiling ethyl acetate. Hérissé's method gave a yield slightly better than that previously obtained.

Possibly the best procedure was a simple modification of the method outlined (Steyn and Rimington, 1935), but even so, the yield fell far short of that theoretically obtainable.

The lead precipitation was carried out in the presence of cadmium nitrate and ammonia and the clarified extract treated as previously described. Having obtained the ether-alcohol filtrate and evaporated off the solvents, a little water was added to the residue followed by much decolorising charcoal and a little calcium carbonate. The mass was then dried as thoroughly as possible, powdered, introduced into a Soxhlet thimble and extracted by hot ethyl acetate. A perfectly colourless ethyl-acetate solution was thus obtained, from which crystals separated on concentrating and cooling. They were recrystallised from absolute alcohol-ether.



*Pinit* the impurity accompanying the glucoside in the final ethyl-acetate solutions was separated by means of its insolubility in boiling absolute alcohol. It crystallised in wedge-shaped prisms and had M.P. 184°. Micro-analysis\* afforded the following figures:

	C	H	N
Found.....	43.41	7.06	Nil
C <sub>7</sub> H <sub>14</sub> O <sub>6</sub> requires.....	43.30	7.22	—

The optical rotatory power was determined in a 2 dm. tube using an aqueous solution:

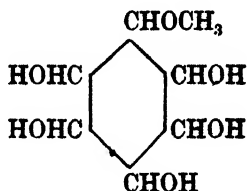
Wt. of substance.....	0.0978 gm.
Volume of solution.....	15 c.c.
Rotation observed.....	+ 0.78°

$$\therefore \left[ \alpha \right]_{\text{D}}^{22} = + \frac{0.78 \times 100 \times 15}{2 \times 9.78} \\ = + 59.8^{\circ}$$

The constants for *pinit* are M.P. 186° and  $\left[ \alpha \right]_{\text{D}}^{25} = + 65.3$ .

The isolated material was found to reduce ammoniacal silver nitrate on heating. *Pinit* occurs in the following plants: *Pinus lambertiana* Dougl.; *Abies pectinata*, D.C. *Cassia angustifolia* Vahl., *Landolphia madagascariensis*, Schum., *Sequoja sempervirens* Endl.

Its constitution is:—



#### ISOLATION OF ACACIPETALIN FROM *Acacia stolonifera*, BURCH.

Qualitative tests performed by Steyn and Rimington (1935) showed that both the fresh leaves and immature pods of *Acacia stolonifera* contained appreciable quantities of cyanogenetic substances.

A larger batch of material was gathered in the same locality (Wonderboompoort, near Pretoria), on 20th April, 1934, when the tree was in the seeding stage. The leaves, when dried and ground, yielded 62 mgm HCN per 100 gms. 1 kg. of the powdered material was exhausted with hot 96 per cent. alcohol in the presence of a considerable quantity of calcium carbonate. The extract was concentrated under reduced pressure and then by the fan to dryness. The residue was taken up in water, filtered, basic lead acetate added and then 20 gm. of cadmium nitrate, dissolved in water, and sufficient ammonia to produce complete precipitation. The liquid was

\* Micro-analyses by Dr. Backeberg, of the University of the Witwatersrand, to whom I wish to express my thanks.

filtered, neutralised by acetic acid and excess of lead removed by hydrogen sulphide. After removal of the lead sulphide and excess of gas, the clear solution was concentrated in presence of calcium

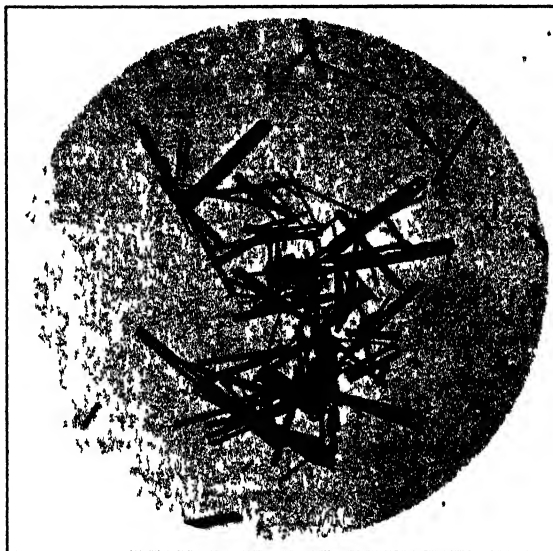


Fig. II (a) — *Acacipetalin* crystallised from abs alcohol-ether  $\times 65$

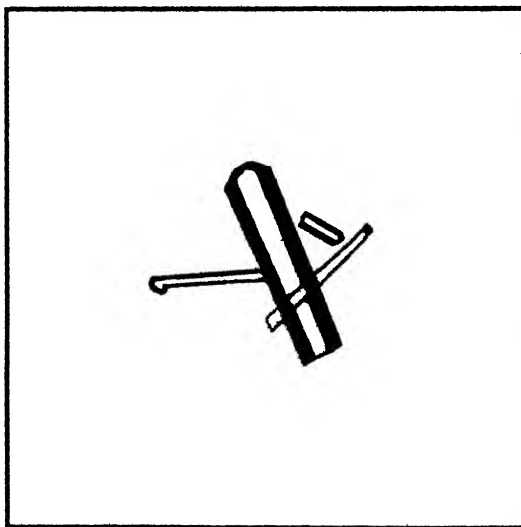


Fig II (b) — *Acacipetalin*  $\times 260$ .

carbonate, first in vacuo and then by the fan, until it formed a thin syrup. A considerable quantity of decolorising charcoal and calcium carbonate were now stirred in until the mass was no longer sticky.

It was spread out in thin layers and dried first in the air and then in vacuo over sulphuric acid. It was finally powdered, introduced into a soxhlet extraction thimble and continuously extracted by boiling ethyl acetate for two days. As a precautionary measure some calcium carbonate was placed in the bottom of each extraction flask.

The pale, straw-coloured extracts were combined and concentrated. On cooling a large crop of crystalline glucoside separated and further crystals were obtained after concentration of the mother liquors. The material was washed with dry ice-cold ethyl acetate and dried in vacuo. Final recrystallisation by the absolute alcohol-ether method yielded an analytically pure substance with M.P. 176-7°. The yield was 1.12 gm. or about 20 per cent. of that theoretically possible.

From a further large batch of young leaves gathered from the same tree on 31st November, 1934 (i.e. in the spring), a yield of 10.69 gm. of Acacipetalin was obtained.

The glucoside crystallised in colourless six-sided prisms (see Figs II) had M.P. 176-7° and possessed a bitter taste. It resembled in all respects Acacipetalin from *Acacia lasiopetala*.

Micro-analysis:—

	C	H	N
Found.....	51.17	6.72	5.35
C <sub>11</sub> H <sub>17</sub> O <sub>6</sub> N requires.....	50.78	6.59	5.43

The optical rotatory power, determined in a 2 dm. tube, agreed with the value (-35.96) previously found for Acacipetalin from *Acacia lasiopetala* (see Steyn and Rimington).

C = 0.25 gm. in 15 c.c. water.

$\alpha = -1.22^\circ$

$$\therefore \left[ \alpha \right]_D^{26} = \frac{-1.22 \times 100 \times 15}{2 \times 25} \\ = -36.60^\circ$$

#### CONSTITUTION OF ACACIPETALIN.

Acacipetalin possesses a somewhat remarkable constitution. On account of the unstable nature of the aglucone, it does not yield, on hydrolysis, an aldehyde or ketone together with hydrogen cyanide as do most cyanogenetic glucosides. Alkaline, followed by acid hydrolysis was found ultimately to provide the key to the solution of its structure. The various steps by which the final conclusion was arrived at are detailed below.

##### 1. Empirical formula.

Micro-analysis of different preparations afforded figures agreeing most closely with C<sub>11</sub>H<sub>17</sub>O<sub>6</sub>N. This was also shown to be the molecular formula from molecular weight determinations (see below).

Such a formula would demand that the aglucone formed when one molecule of glucose and one molecule of hydrogen cyanide are removed should have the composition  $C_4H_6O$ . This represents an unsaturated compound, there being some five or six isomeric possibilities. Had the original formula for the glucoside contained two more hydrogen atoms, making  $C_{11}H_{19}O_6N$ , a saturated aglucone  $C_4H_8O$  would be indicated by suitable calculations. Only three possibilities would then have to be considered, normal and iso-butyl aldehydes and methylethylketone.

Degradation experiments proved conclusively that neither of these three substances was formed so that the formula  $C_{11}H_{17}O_6N$ , can be accepted as correct. The analytical data agree more closely with this than with  $C_{11}H_{19}O_6N$ , thus:—

	C	H	N
$C_{11}H_{19}O_6N$ requires.	50.39	7.31	5.38
$C_{11}H_{17}O_6N$ „	50.78	6.59	5.43
Found...	50.94	6.68	5.45 ( <i>A. lasiopetala</i> )
	51.17	6.72	5.35 ( <i>A. stolonifera</i> ).

Methoxyl groups,  $CH_3O$ , were tested for but found to be absent.

## 2. Molecular Weight.

This was determined in water by the cryoscopic method and found to support the simple formula  $C_{11}H_{17}O_6N$

Weight of glucoside.....	0.250 gm.
Weight of solvent.....	14.9 gm.
Observed depression.....	0.120 ; 0.125
∴ M. Wt. = 258.6 ; 248.3	
$C_{11}H_{17}O_6N$ requires 259	

## 3. Identification of Glucose and Hydrogen Cyanide: enzyme hydrolysis.

0.1460 gm. of Acacipetalin was dissolved in 15 c.c. of water and about 1 mgm. of an active emulsin preparation added. The tube was stoppered, including a piece of sodium picrate paper, and left overnight in an incubator at 37°.

The picrate paper was rapidly turned reddish-brown as hydrolysis proceeded. Hydrogen cyanide was also detected by the prussian blue test.

The hydrolysis mixture was filtered into a 2 dm. tube and its optical rotatory power measured. It was found to be +0.71° and from this the quantity of sugar present was calculated on the assumption that it was entirely glucose.

$$\frac{0.71 \times 15}{52.5 \times 2} = 0.1014 \text{ gm. glucose present.}$$

Theory requires 0.1007 gm.

The osazone was prepared in the usual way and isolated as sheaves of yellow needles; wt. 18 mgm.

It was recrystallised from dilute alcohol-pyridine and obtained in stellate clusters of needles, M.P. 204·5-205°.

Mixed with authentic glucosazone of M.P. 204°, the melting point was undepressed 204·5°.

Micro-analysis:—

	N
Found.....	15·39
$C_{18}H_{22}O_4N_4$ requires.....	15·64

It is thus clear that Acacipetalin contains one molecule of glucose and is a  $\beta$ -glucoside since it is readily hydrolysed by the enzyme emulsin.

#### 4. Detection of acetone among the products of enzymic hydrolysis.

On the expectation that Acacipetalin would yield a simple aldehyde or ketone on hydrolysis, derived from the break-up of a cyanhydrin, attempts were made to isolate this material as the 2:4 dinitrophenylhydrazone.

A solution of 60 mgm. of glucoside in 10 c.c. of water was hydrolysed by emulsin and the mixture distilled from a small distilling flask into an ice-cooled receiver containing a little water. Sufficient hydrochloric acid was added to the distillate to bring to 2N concentration and then 5 c.c. of hot Brady's solution (0·5 gm. 2:4 dinitrophenylhydrazine dissolved in 30 c.c. of 2N hydrochloric acid).

A small quantity of precipitate formed. This was centrifuged down, washed well with 2N acid and then with water and finally recrystallised from hot 60 per cent. alcohol. The material separated in the form of long orange-coloured needles together with plate-like crystals of a lighter colour. This appearance is characteristic of the 2:4 dinitrophenylhydrazone of acetone. The yield was 10 mgm.

The material had M.P. 124°.

Mixed with authentic acetone 2:4 dinitrophenylhydrazone of M.P. 124° it had M.P. 123·4°.

Micro-analysis:—

	C	H	N
Found.....	45·23	4·30	22·87
$C_9H_{10}O_4N_4$ requires....	45·37	4·20	23·53

The substance present was therefore acetone.

Since this result was somewhat surprising, its accuracy was checked by running a control experiment in which emulsin and water were incubated. On distillation no trace of any substance reacting with Brady's reagent could be detected. The acetone had not originated therefore from the enzyme.

A second experiment was performed in which the distillate from a hydrolysis mixture was treated with p-nitrophenylhydrazine. A small quantity of a micro-crystalline p-nitrophenylhydrazone was obtained which when recrystallised from dilute alcohol separated in the form of yellow needles with M.P. 139-144°. Authentic acetone p-nitrophenylhydrazone prepared for comparison had M.P. 144-6° and the mixture melted without depression at 139-145°.

The  $\beta$ -glucoside of acetonecyanhydrin is the well known substance linamarin  $C_{10}H_{17}O_6N$ , whose properties are quite different from those of Acacipetalin. The latter, moreover, contains one more carbon atom in its molecule. The production of acetone on enzymic hydrolysis was at this stage difficult to interpret, but it was noted that the yield was very small and that other substances of an acidic nature were produced. It was considered possible that the cyanide group might be attached to the glucose molecule in the form of glucose-cyanhydrin (as in the naturally occurring cyanogenetic glucoside lotusin), although the ready hydrolysis of Acacipetalin by emulsin rendered this hypothesis somewhat improbable. The aglucone would have to be attached to the glucose in some other way than through the aldehyde group and no compound of such configuration is known to be hydrolysed by emulsin.

Nevertheless, alkaline hydrolysis of Acacipetalin was carried out and heptogluconic acid sought for. No trace of this substance could be isolated.

A further possibility entertained was that the acetone isolated after distillation of the enzymic hydrolysate was being formed from some or other heat-labile precursor. Accordingly an experiment was carried out in which emulsin was added to a solution of glucoside and after the hydrolysis at 37° the solution was filtered and hydrochloric acid and Brady's reagent added without any previous distillation. The 2:4 dinitrophenylhydrazone which separated was small in amount and somewhat difficult to crystallise but was finally identified with certainty as acetone. It crystallised from dilute alcohol in the characteristic manner and had M.P. 122.5°. When mixed with authentic material of M.P. 124°, it had M.P. 119-124°.

### 5. *Tetra-acetylacacipetalin.*

The acetyl derivative of Acacipetalin was next prepared and this shown to have the normal composition, thus demonstrating that Acacipetalin contains only the four free hydroxyl groups of the glucose residue.

0.18 gm. of glucoside was dissolved in a mixture of 0.5 c.c. of acetic anhydride and 1.5 c.c. of pyridine. After five days at room temperature, the mixture was poured into ice water. The precipitated crystalline acetyl compound was washed well with ice water and recrystallised, first from absolute alcohol and then from dilute alcohol. The yield was 0.207 gm. or approximately 70 per cent.

## Micro-analysis:—

	C	H	N
Found.....	54.06	6.24	3.69
The tetra-acetyl derivative			
$C_{11}H_{13}(CH_3CO)_4O_6N$ requires...	53.37	5.90	3.28

Tetra-acetylacacipetalin crystallises in long flattened prisms of M.P.  $104^\circ$  (see Fig. III). It is easily soluble in absolute alcohol and in ethylacetate, insoluble in petroleum ether and in water.



Fig III.—Tetra-Acetylacacipetalin  $\times 200$ .

Emulsin added to a suspension of the substance in water produced no change in 24 hours at  $37^\circ$ , but dilute sulphuric acid at water bath temperature slowly liberated hydrogen cyanide.

The optical rotatory power was determined in absolute alcoholic solution.

Weight of tetra-acetylacacipetalin.....	0.0972 gm.
Volume of alcohol.....	15 c.c.
Rotation observed.....	— 0.21°

$$\therefore \left[ \alpha \right]_{\text{D}}^{26} = \frac{-0.21 \times 15 \times 100}{2 \times 9.72}$$

$$= -16.20^{\circ}$$

#### 6. *Alkaline followed by acid hydrolysis—Isolation of isobutyrylformic acid.*

Preliminary experiments having shown that the products of acid hydrolysis of the glucoside were unusual, no aldehyde or ketone being formed, attention was directed towards the action of alkalis. The glucosidic linkage being fairly stable towards boiling baryta, it is possible to hydrolyse the -CN group to carboxyl -COOH in fairly good yield without removing the glucose residue. Acid hydrolysis then gives an aglucone possessing acidic properties which may be used for its isolation and identification. Since hydrogen cyanide as a decomposition product of acacipetalin had only been determined qualitatively and by its physiological action, the opportunity was taken during the alkaline hydrolysis of isolating and identifying the ammonia formed (as the chloroplatinate) thereby confirming the presence of the -CN grouping.

1.0 gm. acacipetalin was refluxed for nine hours with 10 c.c. of saturated baryta in a flask connected by a ground glass joint to an upright condenser carrying at its upper end a trap bulb containing 2 c.c. of normal sulphuric acid. At the conclusion of the hydrolysis, the cooling water was run out of the condenser and all ammonia driven up into the acid trap. This was emptied into a beaker, a little hydrochloric acid added and then an excess of platinic chloride solution. The separation of ammoniumchloroplatinate commenced immediately and was completed in the ice chest. The bipyramidal crystals were collected on the centrifuge, washed with a little ice-water, dried and analysed with the following result, which confirmed their identity as the ammonium salt.

<i>Analysis.</i>	<i>Pt residue.</i>
Found.....	43.75
(NH <sub>4</sub> ) <sub>2</sub> PtCl <sub>6</sub> requires.....	43.95

The baryta hydrolysate gave no precipitate, under the proper conditions, with Brady's reagent. It was acidified, while still slightly warm, with sulphuric acid, added to produce a final concentration of approximately 2 per cent., the barium sulphate centrifuged off, washed with 2 per cent. acid and the washings and supernatant returned to the flask. The contents was boiled under reflux condenser for two hours. The acid liquid was found to give a crystalline precipitate with Brady's reagent, but this was soluble in sodium carbonate solution.



Before working up the acid liquid, the quantity of glucose present was determined polarimetrically.

Volume of solution.....	55 c.c.
Rotation in a 2 dm. tube.....	+ 1.02°
	$1.02 \times 55$
∴ Quantity of glucose.....	$= \frac{2 \times 52.5}{}$
	$= 0.5343 \text{ gm.}$

This represents a yield of 77 per cent. since 1 gm. of glucoside would yield 0.6950 gm. glucose, assuming that no hydrolysis and destruction had taken place during the boiling with baryta.

The osazone was prepared from an aliquot and found to have M.P. 204°. Mixed with glucosazone (204°) M.P. 204°.

In order to examine the acid hydrolysate for neutral aldehydic or ketonic compounds, it was made slightly alkaline with baryta, filtered and distilled. The distillate was divided into two portions, the one treated with Brady's reagent and the other tested with sodium nitroprusside and ammonia and the iodoform reaction also applied. In neither case was a positive result forthcoming: there was no precipitate on adding Brady's reagent. Acetone was therefore absent from the distillate.

The main solution was now rendered acid with sulphuric acid, barium sulphate removed and the clear liquid extracted repeatedly with ether. The ether layer was washed once with water and then shaken with a little sodium carbonate solution, which immediately removed the material giving the dinitrophenylhydrazone precipitate.

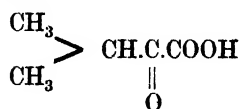
The sodium carbonate extract was aerated to remove ether, hydrochloric acid added to a final concentration of 2N and then a slight excess of hot Brady's reagent. The crystalline precipitate which formed was centrifuged down, washed with 2N acid and then with water and finally recrystallised from hot 60 per cent. alcohol. It was obtained in the form of orange-yellow, square-ended rectangular prisms. They had M.P. 188-190° unchanged by recrystallisation. The material was soluble in sodium carbonate solution, from which it could be reprecipitated in the crystalline condition (M.P. 188-191) by excess of hydrochloric acid, this behaviour strongly suggesting that the material was the 2:4 dinitrophenylhydrazone of an aldehydic or ketonic acid. The supposition was confirmed by analysis, which showed it to be the derivative of a substance having the formula  $C_5H_8O_3$ .

Micro-analysis:—

	C	H	N
Found.....	45.19	4.40	18.40
$C_{11}H_{12}O_6N_4$ requires....	44.60	4.05	18.92

Beilstein (4th Ed.) lists nine isomeric oxo-acids having the formula  $C_5H_8O_3$ , but of these, considering the isolation of acetone from the products of enzymic hydrolysis of the glucoside, as

previously recorded, that which appeared most probable was isobutyrylformic acid ( $\alpha$ -oxo- $\beta$  methylpropan- $\alpha$ -carboxylic acid; dimethylpyruvic acid) possessing the following structure:—



Accordingly, this acid was synthesised, following Franke and Kohn (1899), from isobutyrideneacetone and also by the more acceptable method via isobutyrylcyanide (Tschelinzeff, 1929; Craig 1934) and its 2:4 dinitrophenylhydrazone prepared. This compound crystallised in long, slightly orange-yellow rectangular prisms, which, after repeated recrystallisation from dilute alcohol, had M.P. 190°. Mixed with the material from the glucoside (M.P. 188-190°) the mixture melted without depression at 189-190°, thereby proving the identity of the two materials and establishing the degradation product of acacipetalin as isobutyrylformic acid.

*Synthesis of Isobutyrideneacetone and of Isobutyrylformic acid.*

Some details of the method employed may be reproduced here since Franke and Kohn's paper contains only a very meagre description of the technique they adopted.

57 c.c. of isobutyraldehyde and 46 c.c. of acetone were shaken in a separatory funnel with 100 c.c. of a 10 per cent. aqueous solution of sodium hydroxide. The mixture became hot and developed a pale yellow colour. After standing for several days with occasional shaking, the lower layer was removed and discarded, the oil being washed twice with distilled water, after which it was fractionated and the liquid distilling below 155° discarded. The main fraction passed over at 155-158° (uncor.), after which the temperature rose rapidly. The isobutyrideneacetone so obtained was purified by redistillation. It was colourless but gradually acquired a pale yellow colour on standing, probably owing to polymerisation. The 2:4 dinitrophenylhydrazone was prepared and found to crystallise in long, orange-red prismatic needles of M.P. 163-5°.

<i>Microanalysis.</i>	N
Found.....	18.60
$\text{C}_{13}\text{H}_{16}\text{O}_4\text{N}_4$ requires.....	19.17

For the preparation of the ketonic acid, 10 gm. of isobutyrideneacetone was mixed with about 100 c.c. of water and a cold 1 per cent. solution of potassium permanganate (700 c.c.) slowly run it to the ice-cooled and mechanically stirred mixture. This is considerably less permanganate than was used by Franke and Kohn, but when following their method, a considerable excess remained unreduced and great difficulty was experienced in isolating any isobutyrylformic acid at all. After filtering, traces of unchanged starting materials

which still remained were removed by extraction with ether. The mixture was then rendered strongly acid with sulphuric acid and again extracted by ether. The ethereal solution on evaporation left the ketonic acid as an oil, which was purified by mixing with water, shaking with a little charcoal and filtering. The aqueous solution was used for the preparation of the 2:4 dinitrophenylhydrazone, which had to be recrystallised repeatedly before a satisfactory melting point was obtained. Synthesis via butyrylcyanide yielded pure material at the first crystallisation, M.P. 189-191°.

<i>Micro-analysis.</i>	N
Found.....	18.82
$C_{11}H_{12}O_6N_4$ requires.....	18.92

### 7. Hydrolysis by acid.

The products of acid hydrolysis of the glucoside were next examined. As previously stated it had been found in preliminary experiments that no more than a possible trace of aldehyde or ketone was produced.

0.1 gm. Acacipetalin was refluxed with 10 c.c. of 1 per cent. sulphuric acid for 1 hour. Hydrogen cyanide was evolved. The cooled liquid was then made slightly alkaline with sodium hydroxide and distilled. To the distillate were added hydrochloric acid to 2N and 4 c.c. of hot Brady's reagent. A very slight turbidity formed, which settled into a flocculent precipitate after some hours, and this was centrifuged off and washed. When recrystallised it had M.P. 158-162°, but the quantity was so small that further identification was impossible. Formaldehyde 2:4 dinitrophenylhydrazone melts at 161°, and it is possible that this may have been the material isolated.

The residue in the distilling flask was diluted, acidified and again distilled. The distillate gave no trace of turbidity with Brady's reagent, but it was noticed that it reacted acid to litmus.

The contents of the distilling flask was made up to a solution of 15 c.c. and its rotation determined in a 2 dm. tube.

Rotation observed.....	+ 0.48°
	0.48 × 15
∴ Quantity of sugar as glucose.....	= $\frac{52.5 \times 2}{52.5 \times 2}$
	= 0.0686 gm.
Theoretical yield from 0.1 gm. glucoside..	= 0.0695 gm.

The osazone prepared in the usual way had M.P. 201-5°. Mixed with glucosazone, M.P. 203-5°. In a second experiment, 1 gm. of glucoside was hydrolysed by refluxing for two hours with 25 c.c. of 0.5N sulphuric acid. When cold, the mixture was extracted repeatedly with ether. The ethereal solution on evaporation left a

liquid, acid residue with a butyric-like smell. On titration it neutralised 8.4 c.c. of N/10 sodium hydroxide, and the sodium salt obtained by evaporation to dryness weighed 90.8 gm.

∴ Equivalent weight by calculation.....	86.0
$C_4H_8O_2$ requires.....	88

The sodium salt crystallised in flat irregular plates and was slightly hygroscopic. For further identification the p-toluide was prepared, a control being simultaneously worked up, starting from 80 gm. of sodium isobutyrate.

The sodium salt was transferred into a pyrex test tube (6" × 0.5") hanging by the lip from a small sheet of asbestos, 0.1 c.c. of concentrated hydrochloric acid and 0.2 gm. of p-toluidine were added and the tube heated by a micro-burner at such a rate that the vapours of the toluidine condensed in a ring about half way up the tube. Heating was maintained for one hour. The contents were then extracted by hot absolute alcohol and poured into 10 c.c. of boiling water. The liquid was boiled down rapidly to a volume of about 2 c.c. when a pinch of decolorising charcoal was added and the mixture filtered. The filtrate was concentrated to dryness, extracted by boiling benzene, the pale yellow benzene solution evaporated to dryness and the partly crystalline residue boiled with 2 c.c. of water in successive 1 c.c. portions, the clear colourless aqueous solution being filtered from insoluble tarry impurities and allowed to concentrate slowly in a shallow basin placed in an unexhausted calcium chloride desiccator. A small crop of large lustrous plates separated. These were dried on a porous tile and found to be similar in appearance to those from the control sodium isobutyrate experiment.

The identity of the two p-toluides was proved by melting point determination, thus—

p-toluide of acid from Acacipetalin M.P. 102°

control isobutyr-p-toluide M.P. 102-102.5°

Mixed M.P. 102°.

The products of the acid hydrolysis of Acacipetalin are thus, hydrogen cyanide, glucose and isobutyric acid, with possibly a trace of formaldehyde.

#### THE CONSTITUTIONAL FORMULA OF ACACIPETALIN.

In arriving at a decision concerning the constitution of Acacipetalin the following facts had to be considered:—

- (1) One molecule of glucose was identified quantitatively and qualitatively after enzymic, acid, or alkaline, followed by acid hydrolysis.

OCCURRENCE OF CYANOGENETIC GLUCOSIDES IN S.A. ACACIA.

- (2) No heptogluconic acid could be found as a decomposition product.
- (3) Acacipetalin yields a tetra-acetyl derivative.
- (4) Emulsin readily hydrolyses the glucoside with liberation of HCN.

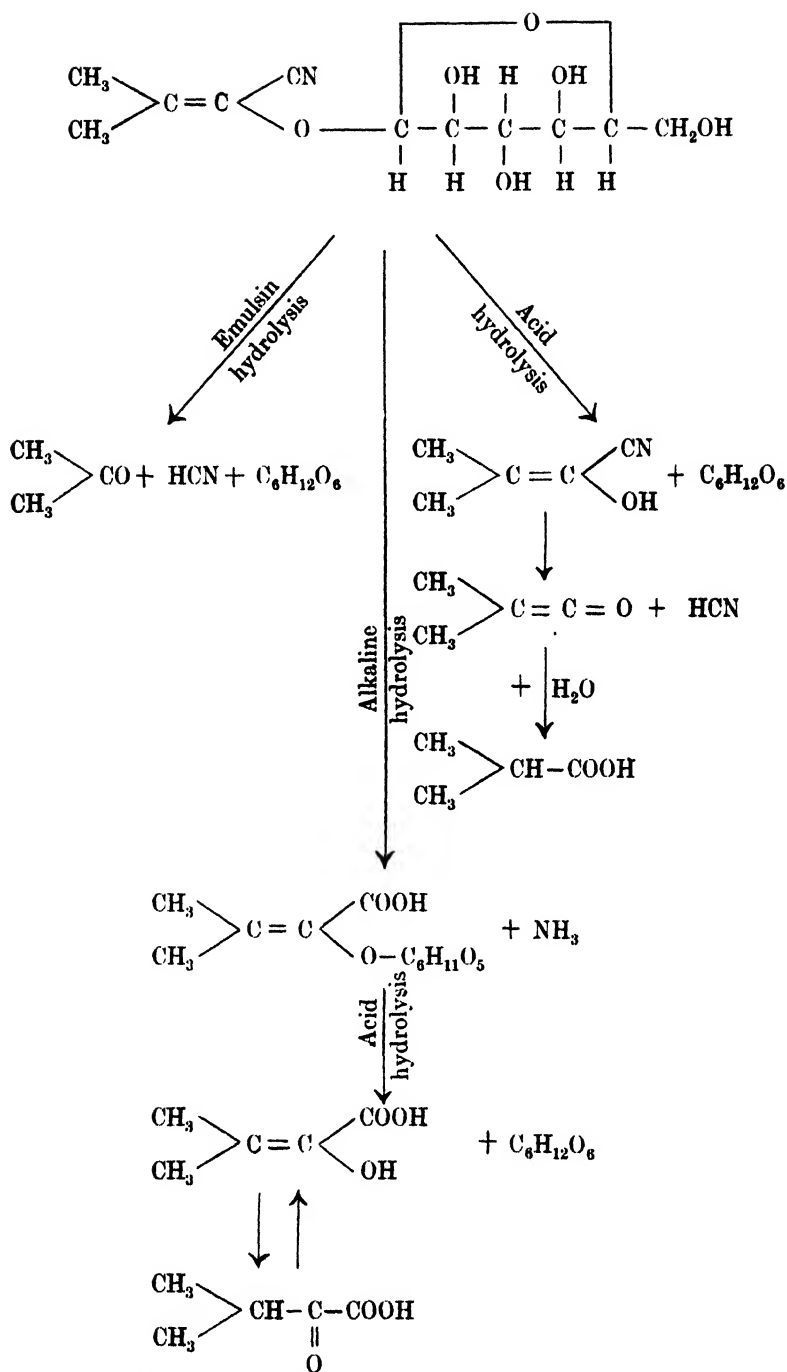
It is clear therefore that Acacipetalin must be a  $\beta$ -glucoside in which the nitrile group resides outside of the glucose residue in the aglucone.

- (5) The aglucone from its derived empirical formula must be unsaturated in character.
- (6) It is probably unstable, breaking up under acid hydrolysis conditions to yield isobutyric acid.
- (7) Alkaline hydrolysis converts the -CN group into -COOH and  $\text{NH}_3$ . When the glucose residue is subsequently removed an  $\alpha$ -ketonic acid is formed.

Usually such procedure leads to the production from cyanogenetic glucosides of  $\alpha$ -hydroxyacids but owing to the keto-enol tautomerism, the ketonic acid may also be regarded as an  $\alpha$ - $\beta$  unsaturated-  $\alpha$  -hydroxy acid.

- (8) Acid hydrolysis would thus lead to a ketene cyanhydrin which would be expected to break up into hydrogen cyanide and the corresponding ketene. Dimethyl ketene is unstable and in contact with water yields isobutyric acid (Beilstein, 4th Ed., Vol. I, Syst. No. 90) which latter was identified after acid hydrolysis of the glucoside.
- (9) The production of acetone in small yield (together with volatile acidic substances) among the products of enzymic hydrolysis is also susceptible of explanation (see below).

It is considered that all reactions of the glucoside can be adequately explained on the basis of the following structure which is therefore proposed as representing the constitution of Acacipetalin.



With regard to the isolation of acetone from the products of enzymic but not of acid hydrolysis, some discussion is warranted since these results are at first sight somewhat surprising.

It must be emphasised that the conditions are very different in the two cases. In the former, dimethyl-ketene appears in an acid medium at the boiling temperature, whilst in the latter it is formed only slowly *in statu nascendi* in a neutral medium at 37°.

The production of acetone from the ketene might occur as the result of an oxidation at the ethylenic linkage. A crude enzyme preparation such as emulsin is likely to contain oxido-reductive ferments in addition to those concerned in the liberation of glucose and production of free HCN from the cyanhydrin. What the action of these might be it is impossible to foretell.

There is evidence from a purely chemical standpoint, however, that ketenes on decomposition yield appreciable amounts of ketones. Thus Hurd and Dull (1932) discussing the preparation of ketenes by the pyrolysis of acylphthalimides state that a certain proportion of acids or their corresponding anhydrides and also ketones were formed in appreciable quantity, notwithstanding the fact that the starting materials had been dried with the utmost care. Thus, for example, among the products of the pyrolysis of propionylphthalimide were found propionic anhydride, diethyl ketone and carbon dioxide. Ketenes being very reactive and unstable materials may, at the moment of their formation, undergo changes in various ways among which must be numbered the production of ketonic bodies.

Attempts to hydrogenate the double bond in Acacipetalin were unsatisfactory. Several trials were made using a colloidal palladium catalyst and hydrogen as in the Paal process. A fairly slow but steady uptake of hydrogen was observed in each case, but the reaction was not quantitative, and it was soon evident that changes of a deep-seated nature were taking place. In the reaction mixture were detected small quantities of glucose and amines but no hydrogen cyanide.

It is clear that hydrogenation in the presence of palladium brings about a fairly extensive destruction of the glucoside; the reactions were much too complicated to be used as evidence of structure (compare Skita, 1909, 1915; Skita and Meyer, 1912; Paal and Gerum, 1909, etc.).

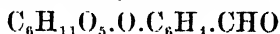
#### . BIOLOGICAL RELATIONSHIPS.

Finnemore and Cox (1930) isolated from the Australian *Acacia* species, *A. glaucescens* and *A. chceli*, the cyanogenetic glucoside sambunigrin. Sambunigrin is a benzaldehydecyanhydrin glucose ether and bears no relationship to Acacipetalin. The South African species of *Acacia* belong to a different group in the genus, bearing true leaves, whilst those investigated by the Australian workers are phyllodineous. This circumstance probably accounts for the very different chemical findings.

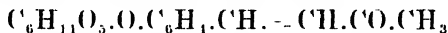
Acacipetalin differs from typical cyanogenetic glucosides only in possessing an unsaturated aglucone which leads to a variety of secondary decomposition products when the ketene is set free by hydrolysis.

Unsaturated aglycones are met with in the glucosides, Gluconapin from *Brassica rapus* and many other mustard oil glucosides. Isobutyl mustard oil occurs in *Cochlearia officinalis*.

Helicin, the glucoside of salicylic aldehyde,



has been combined with acetone (Tiemann and Rees, 1885) to form the unsaturated synthetic glucoside



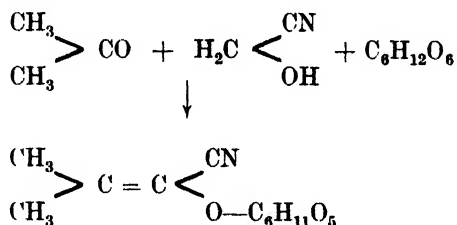
Coniferin, the glucoside of coniferyl alcohol and occurring in conifers, also contains an ethylenic linkage



Syringin is closely similar. Such examples might be multiplied, but sufficient has been said to indicate that unsaturated aglycones are not uncommon although previously not encountered in naturally-occurring cyanogenetic glucosides.

With regard to the possible mode of formation of Acacipetalin in the plant, a tentative suggestion might be made, with all reserve, that acetone and formaldehyde or formaldehydecyanhydrin present themselves as not unlikely starting points.

Such a condensation as that pictured below is not without its counterpart in the chemistry of living materials and is not objectionable from the purely chemical standpoint.



#### SUMMARY.

1. The cyanogenetic glucoside Acacipetalin has been isolated by an improved method from *Acacia stolonifera* Burch. The constants

were found to be M.P. 176-7°;  $\left[\alpha\right]_{\text{D}}^{26} = -36.60^\circ$ .

2. Tetra-acetylacacipetalin has been prepared. It has M.P. 104° and  $\left[\alpha\right]_{\text{D}}^{26} = -16.20$ .

3. Pinit, inositol monomethyl ether, has been identified as a constituent of *Acacia stolonifera*.

4. The constitution of Acacipetalin has been elucidated. It is the glucose ether of dimethylketenecyanhydrin.



5. The facts upon which this conclusion is based are recorded and include the identification of the following breakdown products of the glucoside. On enzymic hydrolysis, hydrogen cyanide, glucose, acetone and acidic substances. On acid hydrolysis, hydrogen cyanide, glucose and isobutyric acid. After alkaline followed by acid hydrolysis, ammonia, glucose and isobutyrylformic acid, isolated as the 2:4 dinitrophenylhydrazone. This latter substance was prepared synthetically and found to have M.P. 190°.

The 2:4 dinitrophenylhydrazone of isobutyrideneacetone crystallises in orange-red prisms and melts at 163.5°.

6. Catalytic hydrogenation using colloidal palladium as catalyst leads to deep-seated changes in the glucoside. Among the reaction products were detected acetone, glucose and amino substances.

7. A comparison with other glucosides containing unsaturated aglycones is made and a suggestion put forward as to the possible mode of origin of Acacipetalin in the plant.

#### ACKNOWLEDGMENTS.

My thanks are due to Dr. D. G. Steyn for his interest in the course of this work and to Dr. Kamerman for some helpful criticism. I also wish to thank Professor Stephen, of the University of the Witwatersrand, and Professor Rindl, of Grey College, Bloemfontein, who kindly supplied me with specimens of pure butyraldehyde and methylethylketone for use as comparison substances.

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## Section IV.

# Toxicology.

STEYN, D. G. ... .. The Toxicity of Trypan Blue ... .. 467



## **The Toxicity of Trypan Blue.**

By DOUW G. STEYN, B.Sc., Dr.Med.Vet., D.V.Sc., Veterinary Research Officer, Onderstepoort.

THIS investigation was prompted by the fact that information was sought in regard to whether, or not, solutions of Trypan Blue became toxic when left standing for a few days before injection.

The following experiments conducted by du Toit (1928) are of interest as far as the toxicity of freshly prepared solutions of Trypan Blue, injected intravenously, are concerned:—

- (a) An eighteen-month old calf received 39 gm. of Trypan Blue (in 1 per cent. solution) in the course of twenty-nine days. The initial dose was 1.5 gm. and the last dose on the twenty-ninth day 4 gm. Trypan Blue. On the fiftieth day after the first injection of Trypan Blue the animal died in a state of advanced cachexia and weakness. It should be mentioned that the animal was suffering from anaplasmosis and that the time of death anaplasmata were still present in the heart blood.
- (b) Calf 316 (2½ years old) received 32.5 gm. (in 1 per cent. solution) Trypan Blue in ten injections in the course of thirty-two days. The initial dose was 1.5 gm. and the last dose 4 gm. The blood of the animal remained normal throughout the experiment. On the 9th, 14th and 20th day of the experiment the animal showed a temperature ranging from 105 to 105.8° F.
- (c) Calf 426 (5½ months old) received 8.5 gm. Trypan Blue in the course of twenty-seven days. The injections were commenced with 0.5 gm. (in 1 per cent. solution) and ended with 0.75 gm. No symptoms of poisoning were noticed, the animal however developed anaplasmosis and gonderiosis. Du Toit considers that the injection of Trypan Blue brought about a relapse of these two diseases, from which the animal had suffered some time prior to the experiment with Trypan Blue.
- (d) Each of two calves 709 and 607 (2½ and 4½ months old respectively) received twenty-six injections of Trypan Blue in a 1 per cent. solution in the course of fifty days. The initial injection was 0.5 gm.; the dose was increased to 0.75 gm., then 1.0 gm., then 1.5 gm., 2.5 gm., 3.0 gm., 4.0 gm., and finally 5.0 gm. Each calf received 36.5 gm. Trypan Blue altogether. No symptoms of poisoning were discernible with the exception of a high temperature (above 106° F.) which occurred on the 9th day after the first injection.

- (e) Calf 424 (2 years old) a carrier of anaplasmosis received fourteen injections of Trypan Blue in a 1 per cent. solution in the course of sixty-seven days, commencing with a dose of 1.0 gm. and ending with 2.5 gm. No symptoms of poisoning except a slight rise in temperature, were discernible.
- (f) Calf 755 (1½ years old), a carrier of anaplasmosis, received fifteen injections of Trypan Blue in a 1 per cent. solution in the course of sixty-seven days, commencing with a dose of 1.0 gm. and ending with one of 3 gm. No symptoms of poisoning were discernible.

Ryrie (1933) who investigated the curative effect of coal-tar dyes in cases of leprosy, states that different samples of the same dye exhibit marked differences in their immediate effect on the patient. He tested the following dyes:—Chrysoidine, Bismark brown, Trypan Blue, brilliant green, Malachite green, Crystal violet, Methyl violet, Auramine, Eosin, Fluorescein, Rhodamine, Methylene blue, Toluidine blue and Indigo Carmine. He describes the toxic effects of these dyes in human beings as follows:—cardiac pain, epigastric palpitation, gastric and rectal irritation, feeble pulse, and signs of shock, and, in severe cases coma and even temporary failure of respiration and loss of radial pulse. He states that solutions tend to become more toxic if left for a few days before injection. He found that a number of dyes, including Trypan Blue, if injected in sufficient quantity show almost at once a selective affinity for the endothelial tissues of the lesions. In most cases patients tolerated 25 cc. of a 4 per cent. solution, whilst some develop symptoms of poisoning. Some patients even tolerated 75 cc. of a 4 per cent. solution. Ryrie states that it is important to filter the solution before injection.

Anderson, Emerson and Fisher (1934) determined the toxic effects of Trypan Blue, Crystal violet and Brilliant green on mice, rats, rabbits and guinea-pigs. Guinea-pigs were found to be more susceptible than mice, rats and rabbits. Three out of five rabbits injected intravenously with 0.15 gm. Trypan Blue per kg. bodyweight in a 5 per cent. aqueous solution succumbed. When administered per os. Trypan Blue is absorbed very slowly and is almost non-toxic. The authors were unable to elucidate the mechanism of the toxic action of the dyes. They suspect Trypan Blue of causing depression of the central nervous system. In all fatal cases there were marked congestion of the lungs. Trypan Blue appeared to cause less damage to the liver than brilliant green and gentian violet. Repeated injections of Trypan Blue into leprosy rats are better tolerated than those of gentian violet and brilliant green.

In another article Anderson, Fisher and Emerson (1934) refer to the toxicity of Trypan Blue and state that "mice survive 300 mgm. per kilogram intraperitoneally, while 400 mgm. per kilogram of a 1 per cent. solution kills all animals. Intravenously the dye is about twice as toxic, being lethal for 3 of 5 mice at 200 mgm. per kilogram. Rats tolerate oral amounts to 1.0 gram per kilogram and do not become blue, indicating no absorption by this route. A sub-cutaneous dose of 400 mgm. per kilogram kills 4 of 5 animals.

Intraperitoneally all rats die when given 350 mgm. per kilogram of a 2 per cent. solution of Trypan Blue. Three hundred mgm. per kilogram is lethal for rats on intravenous administration of a 2 per cent. solution. The dye is slightly more toxic for guinea-pigs killing 3 of 5 animals on subcutaneous injection of 300 mgm. per kilogram, while intraperitoneally half of six guinea-pigs die with 250 mgm. per kilogram of a 2 per cent. solution. Rabbits tolerate larger amounts intraperitoneally surviving 300 mgm. per kilogram, but die when given 400 mgm. per kilogram. Intravenous doses of 100 mgm. per kilogram kill 1 of 5 animals, while 150 mgm. per kilogram of a 5 per cent. solution is lethal for 3 of 5 rabbits."

Gousseff and Sudzilowsky (1934) ascertained the effect of medicinal doses of Trypan Blue manufactured by the Bayer-Meister-Lucius factory) on the horse. They recommend that horses injected intravenously with Trypan Blue should not be worked for a day after the injection. In the case of subcutaneous and intramuscular injections the animals should not be worked for four to five days after the injection.

They state that Trypan Blue had no effect on the bilirubin content and alkali reserve of the blood.

#### ONDERSTEEPOORT EXPERIMENTS.

Rabbit No.	Weight in Kg.	Manufacturer of Trypan Blue.	Amount of Trypan Blue injected intravenously.	Result.
A	2.05	I "Casella" Trademark; manufactured by Leopold Casella & Co., Frankfurt a. M.; Germany	5.0 cc. of a freshly prepared 2 per cent. aqueous solution	No symptoms developed.
B	1.75		10.0 cc. of a freshly prepared 2 per cent. aqueous solution	Slight transient accelerated respiration.
C	2.15		15.0 cc. of a freshly prepared 2 per cent. aqueous solution	Died 36 hours after injection.
D	2.3		5.0 cc. of a 10-day old 2 per cent. aqueous solution	Laboured respiration, which lasted about four hours.
E	2.4		10.0 cc. of a 10-day old 2 per cent. aqueous solution	Died 1 hour after injection.
F	1.6		10.0 cc. of a 10-day old 2 per cent. aqueous solution	Died 14 hours after injection.
G	2.14	II "Ciba" Trademark; Gesellschaft für Chemische Industrie, Basel, Schweiz. (Per Carl Bittmann, 31 Petersgraben, Basel.)	5.0 cc. of a freshly prepared 2 per cent. aqueous solution	No symptoms developed.
H	2.0		10.0 cc. of a freshly prepared 2 per cent. aqueous solution	Died 3½ days after injection.
I	2.14		It was intended to inject 15.0 cc. of a freshly prepared 2 per cent. aqueous solution, but animal collapsed and died after 10 cc. had been injected	Died.
J	1.6		5.0 cc. of a 10-day old 2 per cent. aqueous solution	Slight transient, laboured respiration.
K	1.6		10.0 cc. of a 10-day old 2 per cent. aqueous solution	Pronounced transient, laboured respiration.
L	1.9		15.0 cc. of a 10-day old 2 per cent. aqueous solution	Pronounced transient, laboured respiration.

# TOXICITY OF TRYPAN BLUE.

## ONDERSTEEPOORT EXPERIMENTS (*cont.*).

Rabbit No.	Weight in Kg.	Manufacturer of Trypan Blue.	Amount of Trypan Blue Injected intravenously.	Result.
M	1.6	III Dr. G. Grubler & Co., Leipzig, Germany. (Batch 10,33.)	5.0 cc. of a freshly prepared 2 per cent aqueous solution	No symptoms developed.
N	2.1		10.0 cc. of a freshly prepared 2 per cent. aqueous solution	Transient laboured respiration.
O	2.0		15.0 cc. of a freshly prepared 2 per cent. aqueous solution	Laboured respiration set in immediately after injection. Animal died about 12 hours after injection.
P	1.75		5.0 cc. of a 10-day old 2 per cent. aqueous solution	Died 3 hours after injection.
Q	1.9		10.0 cc. of a 10-day old 2 per cent. aqueous solution	Died 2 hours after injection.
R	1.6		It was intended to inject 15.0 cc. of a 10-day old 2 per cent. aqueous solution. When 12 cc. were injected, severe convulsions set in and at 14 cc. the animal collapsed and died.	

Solutions of the above brands of Trypan Blue were prepared and injected immediately. The remaining quantities of the solution were injected after having been allowed to stand for ten days in flasks fitted with dry plugs of cotton wool. Trypan Blue I solution was violet and not blue in colour. The specimens of Trypan Blue used in the author's experiments were taken from the original containers issued by the respective manufacturers. The solutions were injected at the same rate into all the animals, namely, 5 c.c. per minute.

From the above table it is evident that the solutions of Trypan Blue I and III markedly increased in toxicity after having been left standing for ten days, whilst this was not the case with the solution of Trypan Blue II.

### SYMPTOMS OF POISONING.

After intravenous injection of toxic and lethal amounts of Trypan Blue the rabbits developed the following symptoms: Laboured respiration, accelerated and weak heart-action, and convulsions. Those animals which survived for a while were in a paralytic state after the attack of convulsions had passed off. In peracute cases of poisoning methaemoglobinaemia was present and was detectable both macroscopically and spectroscopically.

The autopsy revealed no lesions of a characteristic type.

### SUMMARY.

It has been definitely proved that two out of three brands of Trypan Blue tested markedly increased in toxicity after the solutions had been left standing for ten days before injection. It is therefore obvious that only freshly prepared solutions of Trypan Blue should be used.

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## Section V.

# Mineral Metabolism and Nutrition.

MYBURGH, S. J. ... ..	The Carotene Content of some South African Feeds ... ..	475
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## I. The Carotene Content of Some South African Feeds.

By S. J. MYBURGH, M.Sc.Agric., Dept. of Biochemistry,  
Onderstepoort.

### (GENERAL OUTLINE.

CAROTENE, belonging to the group of carotinoids—yellow, fat-soluble pigments, of which there are no less than twelve different ones known—has the most extensive occurrence of all natural pigments. Three of the carotinoids, namely, the well-known Lycopin—characteristic pigment of the tomato—and the two isomers,  $\alpha$ -Carotene and  $\beta$ -Carotene, each having the general formula  $C_{40}H_{56}$ , are in character hydrocarbons. The other carotinoids, which are oxygen-containing derivatives of Carotene or Lycopin, have the characteristics of organic acids, with a molecular formula of  $(C_{40}H_{50}O)_n$ —commonly known as “Phytoxanthines”.

The study of the Carotinoids was first started in 1831 by Wackenroder and later in 1837 by Berzilius. Working on leaf-pigments, they discovered that the pigments were closely associated with fats and oils—hence the name Xanthophyll was given to the yellow pigment of leaves by Willstätter (1910). Other workers also gave the carotinoids their attention: Karrer and Solaman (1927) isolated Crocetin ( $C_{40}H_{54}O_4$ ); Zechmeister and Chlcnoky (1927) isolated Capsanthin; Karrer and co-workers (1929, 1930) isolated Zeaxanthin; Kuhn and co-workers (1931) isolated Violaxanthin and Zaxanthin.

All the carotinoids are practically insoluble in water, and easily soluble in fats and oils. They have the characteristic of fat-pigments (Lipochromes) and are therefore fairly common in nature. As regards solubility in the ordinary organic solvents, there are two distinct groups of carotinoids, namely the hydrocarbons (Carotene and Lycopin)—soluble in petroleum ether, benzine, but insoluble in methyl- and ethyl alcohol. The phytoxanthins (Leaf-xanthophyll, Zeaxanthin and others) are insoluble in petroleum-ether and benzine, but soluble in methyl- and ethyl alcohol. Hence the carotinoids can be fractionated into Carotene and Lycopin on the one hand and the phytoxanthins on the other hand, as was shown by Willstätter. Most of the carotinoids are easily oxidised and become bleached under influence of oxygen. Crocetin, Bixin and Azafrin are exceptions (Karrer). The rate of oxidation depends largely on the state of purity of the pigments. Thus a very pure carotene will only absorb oxygen after ten or more days, whereas the

impure form (with traces of iron salts usually) may undergo oxidation within a few hours. Furthermore, the phenols, hydrochinon, etc. strengthen the pigment against oxidation. This, possibly, also happens in the plant-cell, whereby the pigment is fortified.

The carotinoids show intensive halochromeric phenomena with strong acids (sulphuric-, hydrochloric-, trichloroacetic acids) and chloroform solutions of carotinoids with water-free antimony trichloride. The resulting colours vary from blue to violet, and blue to blue-green; the intensity of colour varies. Rosenheim (1925) found, by the use of the tintometer, comparative values for the intensity-mixtures. These halochromeric pigments are very unstable.

Carotene, which occurs most extensively in green plants, also as the pigment of fat, milk, blood serum, liver, corpus luteum, was first discovered by Wackenroder (1831). Arnaud (1886) showed its presence in green leaves of plants, and Willstätter (1910) gave it the formula  $C_{40}H_{56}$ . It was furthermore found in fruits and flowers; in the pollen of the bud.

It was isolated in crystalline form from carrots and green leaves. Fresh green leaves may contain 0.1–0.3 mgm. Carotene per 100 grms.

Kuhn and Lederer (1931), Karrer and co-workers (1930) showed that carrot-carotene consist of two components, namely  $\alpha$ -Carotene (optically active) and  $\beta$ -Carotene (optically inactive).

Carotene extracted from spinach, nettles, as also from most green plants, proved to be mainly the  $\beta$ -form, according to Karrer (1932).

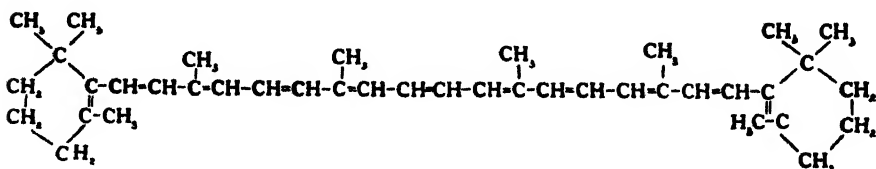
Both isomeric forms of Carotene are soluble in organic solvents, for example in petroleum-ether; but the solubility of these two differ so slightly as to give difficulty in the crystallization process of separation.

The best method of fractionation is by the chromatograph method of Tswett (1911).

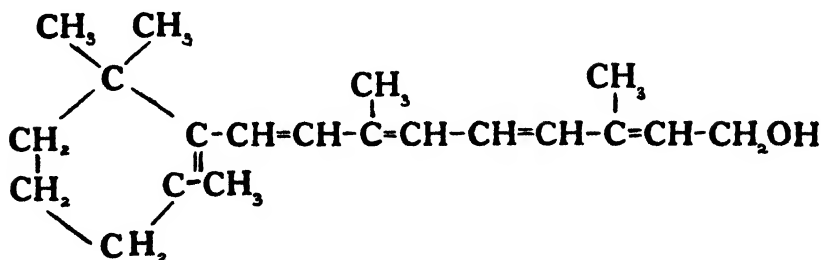
$\beta$ -Carotene has a higher melting point ( $182^{\circ}$  C.) than  $\alpha$ -Carotene ( $172^{\circ}$  C.).

Crystals of Carotene appear red, but solutions of Carotene, in petroleum-ether for example, are deep yellow.

Halogens have only a slight effect on carotene, but iodine form either the tri-iodide ( $C_{40}H_{56}I_3$ ) or di-iodide ( $C_{40}H_{56}I_2$ ). Karrer (1932) gives  $\beta$ -Carotene the following structural formula:—



He furthermore considers  $\beta$ -Carotene the precursor of Vitamin A, which is formed by hydrolysis:—



Apart from cod-liver oil and other liver oils as a source of Vitamin A in biological material, the Carotene of the plant is the natural source of Vitamin A for herbivora. Numerous investigations prove that animals on a diet deficient in Carotene show A-avitaminosis and the young that are dependent on the milk supply of the mother, show stunted growth.

Plants vary in their Carotene content according to species, stage of growth, and portion of plant selected. Lucerne in young, green stage is a rich source of Carotene for Vitamin A, becoming poorer as the plant matures; the leaves are richer than the flowers, stems and seeds. In some plants at the mature stage there is a serious depletion of Carotene; even Carotene-rich plants show only a very small amount at maturity.

Furthermore, hays may also be low in Carotene content; the retention and preservation thereof depending to a great extent on the process of drying and curing. Carotene is easily oxidised by enzymes contained in the plant material, hastening deterioration, as shown by S. Hauge (1935). In the drying and curing of lucerne for hay, the slower the process, the better the oxidation of Carotene, which, therefore, results in a loss of Carotene to animals fed on such hays.

Mechanical drying and curing of lucerne for hay, apart from being time-saving, is far superior to sun drying from the point of view of Carotene-preservation.

In South Africa, where sun drying is the main process in use, loss of Vitamin A may prove to be a matter for consideration, as most of our sun-dried hays are low in Carotene.

#### METHOD OF ANALYSIS OF FEED FOR CAROTENE.

Biological tests with Carotene have often proved unsatisfactory, reproducible results being often unobtainable; a further factor is time.

It has been found by Lathbury and Greenwood (1934) that some oils are beneficial and others not,—causing a loss of Carotene in the latter case,—when used as solvents. Coconut oil is a better solvent, than for example arachis oil. Hence difficulties in biological experimental work have been encountered.

Lately chemical methods for the analysis of material for Carotene have been developed. Guilbert (1934) in working on this subject, employs a colorimetric method for the quantitative expression of Carotene, and the colorimetric work is based on the use of the Colour Standard (International Dye Standard) of Sprague (1928).

In the analysis of various feeds and fodder in this laboratory, the method as given by Guilbert was closely followed. Duplicates of a satisfactory value are obtainable.

The Method of Extraction suggested by Guilbert, produced here :

1-5 gms. of the sample, in finely cut state, was weighed out, transferred to an Erlenmeyer flask and 20 c.c. of freshly prepared saturated solution of potassium hydroxide in ethyl alcohol were added for each gram of sample taken. The flask was fitted to a reflux condenser and the contents gently boiled on a steambath for 30 minutes. Fats and chlorophylls became saponified. The contents of the flask were cooled, 50-100 c.c. ethyl ether added, shaken for a few minutes, the sediment allowed to settle and the ether-alcohol mixture decanted into a large separatory funnel. The flask was washed with small quantities of ether and these were added to the contents of the funnel. The process was repeated until fresh ether when used for rinsing, was colourless (usually 200-250 c.c. ether were necessary for each extraction).

About 100 c.c. (or more) of distilled water were poured down the sides of the funnel through the ether-alcohol solution. The flavones separated and could be drawn off. The solution in funnel was repeatedly washed, drawing off the bottom layer each time. The waste was tested with phenolphthalein to be sure that all alkali had been washed out of the ether-solution.

The ether solution, which contained the yellow pigments Carotene and Xanthophyll, was transferred to a flask and all traces of ether expelled.

The residue was then taken up in 30-40 c.c. petroleum-ether and transferred to a separatory funnel. The Xanthophyll was extracted from the ether solution and separated from Carotene by first shaking with 85 per cent.—and finally with 90 per cent. methyl-alcohol. The lower layer (methyl-alcohol) containing the dissolved Xanthophyll, was separated from the ether solution containing the dissolved Carotene. After 5-6 washings of the ether layer, the methyl-alcohol layer was found to be clear; the last traces of methyl alcohol were rinsed out with small quantities of distilled water.

The ether solution was next dried in contact with about 5 gms. of anhydrous sodium sulphate; it was then poured off, and the salt washed with fresh petroleum ether, until clear—the washings added to the rest of the Carotene-ether solution. The ether solution was poured into a measuring flask, and made up to volume (namely 50 c.c. mark) with petroleum ether. The volume was noted.

The flask was next well shaken and the strength of the Carotene-colour compared against a dye standard in the colorimeter.

A stock solution of this dye standard contained:

Napthol yellow, 3.06 grams;  
Orange G. Crystals, 0.45 grams.

The dyes were dissolved in 1,000 c.c. distilled water. The solution is stable and will keep well in a stoppered flask in the dark.

The standard for the colorimetric work was prepared from the above stock solution, by diluting 50 c.c. of the stock solution to 1,000 c.c.—this colour strength of Dye is equivalent to 2.7 mgm. Carotene (Guilbert).

*Calculations:*

$$\frac{\text{Depth of Standard}}{\text{Colorimetric Reading}} \times \frac{100}{\text{Grams Sample}} \times \frac{\text{Total volume of unknown}}{1,000}$$

$\times$  Value of Standard in mgm. Carotene (2.7) per 1,000 c.c.

= Mgm. Carotene per 100 grams Sample.

TABLE A.

RELATIVE  $\beta$ -CAROTENE VALUES OF VARIOUS FEEDS.

(Value of Dye Standard used = 2.7 mgm. per cent. Carotene.

Description of Sample.	Weight of Sample. (Wet basis) grams.	Moisture Content %	Mgm. % Carotene on Abs. dry basis.	Mgm. % Carotene on fresh basis.
<i>Fresh, young, green Lucerne</i> (whole plant, leaves and stalks).....	15	77.5	40.0	9.0
<i>Fresh, green, Sudan Grass</i> , late stage— (whole plant, leaves and stalks).....	15	79.1	13.5	2.8
<i>Fresh, green Maize plants</i> , late stage— (whole plants, leaves and stalks).....	30	74.0	3.4	0.9
<i>Fresh, green Barley plants</i> (whole plants, leaves and stalks).....	25	79.0	17.1	3.5
<i>Mature grass</i> cut for hay (Armoedsvlakte— Camp A—June, 1935). (Sun dried)....	100	—	—	0.62
<i>Mature Grass</i> cut for hay (Armoedsvlakte— Camp B—June, 1935. (Sun dried)....	100	—	—	0.09



# CAROTENE CONTENT OF SOME S.A. FEEDS.

## TABLE B.

RELATIVE  $\beta$ -CAROTENE VALUES OF FODDER-HAYS.  
(Value of Dye-Standard for Carotene = 2.7 mgm. per cent.)

Description of Sample.	Weight of Sample. (Air dry basis). grams.	Moisture Content. %	Mgm. % Carotene on Abs. dry basis.	Mgm. % Carotene on natural basis.
<i>Lucerne Hay</i> , sun-dried, well-cured (Onderstepoort).....	30	7.6	0.92	0.80
<i>Teff Hay</i> , well-cured (Onderstepoort)....	20.4	1.0	2.50	2.30
<i>Mature Grass Hay</i> , sun dried, well-cured (Onderstepoort).....	100	8.3	$\pm 0.02$	$\pm 0.018$
<i>Yellow Maize Seed</i> —Finely ground.....	100	9.4	0.74	0.67
<i>White Maize Seed</i> —Finely ground.....	100	—	—	trace
<i>Samp</i> —Finely ground (maize endosperm).....	100	—	—	negative
<i>Pig Feed</i> —Mixture of samp and meatmeal (9:1).....	100	—	—	0.047
<i>Maize Meal</i> (yellow).....	100	—	—	0.25
<i>Bran</i> (Wheaten).....	100	—	—	<0.01
<i>Barley Meal</i> .....	100	—	—	<0.01

## TABLE C.

RELATIVE VALUES FOR CAROTENE OF VELD GRASS SAMPLES TAKEN AT DIFFERENT SEASONS FOR TWO SUCCESSIVE YEARS (ARMOEDSVLAKTE).

Sample. Month of Collection.)	Season.	Mgm. % Carotene (on Abs. dry basis).	Mgm. % Carotene (on Air dry basis).
SAMPLE I..... October, 1933.....	Spring	0.11	0.11
SAMPLE II..... January, 1934.....	Summer	1.85	1.71
SAMPLE III..... April, 1934.....	Autumn	0.98	0.92
SAMPLE IV..... July, 1934.....	Winter	0.26	0.24
SAMPLE V..... October, 1934.....	Spring	0.61	0.57
SAMPLE VI..... January, 1935.....	Summer	2.64	2.45
SAMPLE VII..... April, 1935.....	Autumn	4.66	4.32
SAMPLE VIII..... July, 1935.....	Winter	0.23	0.22

## DISCUSSION.

Samples of some of the feed and fodder materials fed to farm animals at Onderstepoort, were analysed for Carotene.

It is evident from Table A that lucerne in young stage of growth is a rich source of carotene.

The green Sudan grass and green Barley, though cut at a fairly late stage of growth (seeding stage) still had appreciable amounts of Carotene. The low Carotene content of the sample of maize plant was probably due to the late stage of growth of this material.

In the case of grass hay the Carotene content ranged from 0.1 to 0.6 mgm. per 100 grams dry material. Hays, in general (Table B) are variable in their Carotene content, the amounts depending on the process of drying and curing. Teff hay gave as much as 2.3 mgm. Carotene per 100 gms. dry material. Probably this teff was cut for hay at a favourable growth-period and was dried and cured under conditions which were favourable for the preservation of its Carotene.

Well-cured, sun-dried lucerne hay was low, probably due to considerable loss of Carotene during the drying and curing processes, as was found to be the case by S. Hauge (1935) in experimental work.

Some mature grass when cut for hay proved to be as low as 0.02 mgm. Carotene per 100 gms. dry material. This hay, even if fed liberally cannot serve as an adequate source of Carotene, if no green material or other food, containing Carotene is given; animals will in time suffer from the effects of Vitamin A deficiency.

Of the maize (seed), the yellow variety, though low in Carotene, was much higher than the white variety.

Analysis of veld grass samples from Armoedsvlakte, Bechuana-land for different seasons of two successive years are tabulated in Table C. For a greater part of the year the natural veld-grass at Armoedsvlakte is apparently low in Carotene. In 1934-35 this was very pronounced during the dry period (winter and spring); in the summer, when copious rains fell (summer-rainfall area) there was new growth and the Carotene of samples taken during that period showed a gradual increase, reaching its maximum in Autumn. Towards winter there was a decline which might continue into Spring or until new growth appeared.

As Carotene is the precursor of Vitamin A, which is essential for the growing animal, as also for normal reproduction, good health and high vitality, it would appear that the danger of an A. avitaminosis during the dry periods of the year in areas like Armoedsvlakte, which are common in other parts of the Union, cannot be excluded. From work which is proceeding it may even be tentatively concluded that such deficiency does exist at times for normal growth and maintenance. Furthermore, hays cured under prevailing conditions and fed back to animals during such period of food scarcity might not contain adequate quantities of Carotene to make good the deficiency in the natural pastures.

The Vitamin A requirements of animals, except pigs—which according to Dunlop (1935) require daily per 100 lb. live-weight approximately 4 mgm. Carotene, are uncertain, but with data that winter pastures in summer-rainfall area are usually low in Carotene, the problem of A-avitaminosis during dry periods, especially under ranching conditions, is well worth further investigation especially in the light of work of Guilbert and Hart. Such work has been started and will be reported on in due course.

#### SUMMARY.

1. Details are given of the method employed at this Institute for the determination of Carotene in some animal foodstuffs.

2. The Carotene content of pasture diminishes rapidly as the pasture matures and becomes dry during winter or during dry periods of drought.

3. The Carotene content of the Cereals included in the determinations is low, yellow maize being the highest and the sample in question containing 0.74 mgms. Carotene per 100 gms. dry material.

4. The existence of an A-avitaminosis in stock entirely dependent on natural pastures during periods, when only dry pasture exists, is discussed.

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## Section VI.

# Physiology.

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## **Studies on the Alimentary Tract of the Merino Sheep in South Africa, II. Investigations on the Physiology of Deglutition, II.**

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In a previous paper on this subject the authors (1933) recorded the results of their investigations into the possibility of stimulating reflex closure of the oesophageal groove, for the purpose of dosing sheep directly into the abomasum. The bulk of the work done consisted of attempts to produce such stimulation by means of sodium salts as Wester had successfully done in the case of cattle, and to find out which factors were limiting or opposing this action of the sodium-ion in sheep. However, since the results were rather inconclusive and the main object had been that of finding a way to administer anthelmintics into the abomasum, it was decided to follow up indications of successful stimulation by a few other chemicals which had been used and to try others if necessary.

Two sheep which had been dosed with a solution containing nicotine and copper sulphate, had swallowed this into the abomasum. A repetition of this test again gave the same result. A test was then made on two sheep with nicotine alone, but this failed to produce the reflex. Before copper sulphate alone was tested a paper by Ross (1933) appeared, in which he recorded successful results with copper sulphate as stimulant. This author used 1 per cent. and 2 per cent. solutions, giving 30 c.c. of these with 10 c.c. of a red stain and he found no significant differences between the results obtained with sheep starved up to 48 hours and unstarved sheep. Of 84 sheep stimulated with 2 per cent. copper sulphate 79 swallowed into the abomasum, and similarly 11 of 12 sheep stimulated with a 1 per cent. solution.

Our results with copper salts which are given below, indicate that reflex closure of the oesophageal groove can be stimulated in this way, but that there are certain limiting factors, and it was our intention to elucidate the conditions under which the best results can be obtained. While the work is still in progress, it has reached a stage at which publication of our results is warranted, particularly also because methods of treatment against gastro-intestinal worms of sheep are now being recommended on this basis.

The sequence of the records of experiments as given below does not coincide with the chronological order in which the tests were made, but was arranged to give a systematic review of the results obtained. Moreover, these experiments were run more or less

parallel with investigations on the chemotherapy of oesophagostomiasis, frequently being influenced by results obtained in that work (see paper by Mönnig in this issue).

### 1. EFFECT OF STARVATION.

Sheep 1-6 were not starved. Sheep 7-14 were kept from food and water for 24 hours.

All the sheep were dosed through a funnel, to which was attached a short length of rubber tubing provided with a metal nozzle, which was placed in the mouth. The drench consisted of 30 c.c. 2 per cent. copper sulphate mixed with 10 c.c. 4 per cent. lithium carmine, or in some cases 4 per cent. erythrosin was used.

TABLE I.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
Unstarved 1.....	8	good	semi-solid	(50·50)
2.....	8	"	fluid	99·1
3.....	8	"	semi-fluid	(10·15)
4.....	2	"	"	20·80
5.....	8	"	semi-solid	90·10
6.....	8	"	"	10·90 (2/2)
Starved 7.....	8	"	fluid	0·100
8.....	8	"	"	5·95
9.....	8	"	"	5·95
10.....	2	"	"	80·20
11.....	2	"	"	0·100
12.....	2	"	"	5·95
13.....	8	"	semi-solid	100·0
14.....	8	"	fluid	(40·60) (2/5)

Under the heading "Result" are given the estimated percentages of the drench which reached the rumen and reticulum (on the left) and the abomasum (on the right). Sheep 3 did not swallow well. Discarding the results of sheep 1, 3 and 14, it is seen that of the unstarved group two swallowed to the rumen and two to the abomasum and of the starved group respectively two and five.

The following sheep were similarly treated, but the copper sulphate solution was administered first, followed immediately by 10 c.c. 4 per cent. erythrosin.

TABLE II.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth			
Unstarved 15.....	2	good	semi-solid	5·95
16.....	2	"	semi-fluid	2·98
17.....	2	"	"	10·90
18.....	2	"	"	0·100 (0/4)
Starved 19.....	2	"	"	2·98
20.....	2	"	"	0·100
21.....	2	"	semi-solid	5·95 (0/3)

The above results, as well of those of all the following tests in which the sheep were not starved, indicate that starvation is not an important factor in bringing about the reflex. In our previous work and also in the present series of tests it was found that fluidity of the ruminal contents favours the establishment of the reflex, and for that reason the sheep had in previous tests frequently been given a drink immediately before being treated. It had, moreover, been observed that long starvation (36-48 hours) tends to produce fluid ruminal contents, and this was also noted by Ross (1931 and 1934), whose findings, that sheep under such conditions readily swallow to the abomasum without stimulation, fully agree with our experiences.

Apart from the fact, however, that sheep suffering badly from worms, particularly *Oesophagostomum columbianum*, cannot safely be starved for lengthy periods, starvation is undesirable for other reasons which have already been discussed by one of us in previous papers (Mönnig 1929a, 1929b, and 1933). It appears, therefore, that sufficient attention has been given to this aspect of the problem and that starvation should not be practised any longer in the dosing of sheep for worms. In the following tests all the sheep were therefore allowed food and water up to the time of treatment.

## 2. METHOD OF ADMINISTRATION.

Stiles (1901) dosed sheep with fluids through a funnel with rubber tubing and a metal nozzle, which was placed between the hind teeth, and he arrived at the conclusion that this method of drenching stimulated the animals to swallow into the abomasum, but that the position in which the sheep was held influenced the result, the most favourable position being that in which the sheep stood normally on its feet. In the above recorded tests the sheep were drenched in this way, but the results were probably not influenced by the method of administration since at least as good results were later obtained by other methods.

TABLE III.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
(a) 30 c.c. 3% CuSO <sub>4</sub> mixed	with teaspoonful red lead	oxide.		
22.....	4	good	semi-solid	2.98
23.....	2	"	"	98.2 (1/1)
(b) 30 c.c. 5% CuSO <sub>4</sub> with te	aspoonful red	lead oxide.		
24.....	2	good	semi-solid	0.100
25.....	2	"	"	15.85 (0/2)
(c) 30 c.c. 3% CuSO <sub>4</sub> , then r	ed lead oxide	by spoon.		
26.....	2	good	semi-solid	2.98
27.....	2	"	"	0.100
28.....	2	"	"	0.100
29.....	2	"	fluid	(50.50) (0/3)
(d) Teaspoonful 5% CuSO <sub>4</sub> b	y spoon, then	n red lead oxide.		
30.....	2	good	fluid	10.90
31.....	2	"	"	0.100 (0/2)
(e) 20 c.c. water with red lead	oxide.			
32.....	2	good	fluid	99.1
33.....	2	"	semi-solid	(50.50) (1/0)



In Table III are given the results on further tests with regard to this point. The fluids were administered to sheep 22-29, 32 and 33 according to the method of Stiles.

Unfortunately sheep 33 must be discarded and the number of sheep in group (e) should have been larger, but the results, particularly group (d), as well as the results of all the following tests, show that the method of administration according to Stiles is not an important factor.

All the above results (Table I and II) seem to indicate that the reflex is stimulated very rapidly once the stimulant reaches the pharynx, but that to administer the full quantity of a drug into the abomasum it is desirable to give the drug after the stimulant and not mixed with it as the first gulp swallowed may pass into the rumen. This was further confirmed by subsequent tests and experiences. Points of importance arising from this conclusion are the time required for the reflex closure of the oesophageal groove to occur and the duration of the reflex.

### 3. RAPIDITY OF REFLEX CLOSURE.

In general it may be stated that the impression obtained in these tests is to the effect that the reflex is established immediately on stimulation, i.e. the moment a solution of the stimulant touches the mucosa of the pharynx. In order to test this point further the following sheep were dosed with a mixture of powdered copper sulphate and red lead oxide. The saliva would have to dissolve some copper sulphate before this could produce the stimulus.

TABLE IV.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
(a) Powder placed on back of tongue (swallowed immediately).	Months.	lowed immed	ately).	
34.....	12	poor	semi-fluid	10.00
35.....	12	"	"	100.0
36.....	12	"	"	100.0 (2/1)
(b) Powder placed on tip of tongue (swallowing delayed).		wing delayed).		
37.....	12	poor	semi-fluid	0.30
38.....	12	"	"	0.100
39.....	12	"	"	1.99 (0/3)
(c) Powder placed into cheek (swallowing delayed).		delayed).		
40.....	12	poor	semi-fluid	0.100
41.....	12	"	"	0.100
42.....	12	"	"	0.100 (0/3)

It is seen that where a slight delay in swallowing occurs—even in sheep 34 this probably was the case—the stimulus is given and the drug passes to the abomasum.

### 4. DURATION OF REFLEX.

It is important to know how long the groove will remain closed in order to allow dosing into the abomasum with large quantities of

fluid or even with small doses of drugs. The time elapsing between the administration of the stimulant and the indicator, red lead oxide, was carefully timed by means of a stop-watch.

TABLE V.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
(a) 50 c.c. 2% CuSO <sub>4</sub> mixed	with red lead oxide.			
43.....	4	good	semi-solid	0·100
44.....	4	"	"	1·99
45.....	4	"	"	5·95 (0/3)
(b) 10 c.c. 5% CuSO <sub>4</sub> , indicator	after 10	seconds.		
46.....	2	good	fluid	0 100
47.....	2	"	"	2 98 (0/2)
(c) 50 c.c. 2% CuSO <sub>4</sub> , indicator	after 15	seconds.		
48.....	4	good	semi-solid	0·100
49.....	4	"	"	5 45 (0/2)
(d) 50 c.c. 2% CuSO <sub>4</sub> , indicator	after 20	seconds.		
50.....	4	good	semi-solid	5 95
51.....	4	"	"	100 0
52.....	4	"	"	5·95 (1/2)
10 c.c. 5% CuSO <sub>4</sub> , indicator	after 20	seconds.		
53.....	4	poor	semi-fluid	60 0
54.....	4	"	semi-solid	50 30
55.....	4	"	semi-fluid	60 0 (3/0)
(e) 50 c.c. 2% CuSO <sub>4</sub> , indicator	after 30	seconds.		
56.....	4	good	semi-solid	95 5
57.....	4	"	"	5 95
58.....	4	"	"	98 2 (2/1)
10 c.c. 5% CuSO <sub>4</sub> , indicator	after 30	seconds.		
59.....	4	poor	semi-solid	0·100
60.....	4	"	solid	0 100
61.....	4	poor	semi-solid	60 0
62.....	2	good	fluid	100·0
63.....	2	"	"	0 100 (2/3)

The tests with 5 per cent. CuSO<sub>4</sub> in groups (d) and (e) were performed later than the rest, after it had been found that the stronger solution produces better stimulation under certain circumstances (see section 5 below). It may be concluded that reliable results can be obtained up to 15 seconds after stimulation, but that after 20 seconds the groove is no longer closed in all cases with the concentration of the stimulant used and taking into consideration the condition of the sheep (section 6) and the consistency of the ruminal contents.

### 5. CONCENTRATION OF STIMULANT.

The work done under this heading was greatly influenced by the results obtained in dosing sheep for *Oesophagostomum columbianum* under field conditions. At first 2 per cent. CuSO<sub>4</sub> was used in many of these therapeutic experiments, until it became clear that the stimulation was not satisfactory. This was followed by 5 per cent. and later 10 per cent. solutions, and the results of stimulation were checked in the degutition tests reported here. In the following tests the red lead oxide was given immediately after the stimulant.

## STUDIES ON THE ALIMENTARY TRACT OF MERINO SHEEP IN S.A., II.

TABLE VI.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
(a) 10 c.c. 0.1% CuSO <sub>4</sub> .	Teeth.			
64.....	6	good	semi-solid	100.0
65.....	6	"	"	0.100
66.....	6	"	"	60.30 (2/1)
(b) 10 c.c. 0.25% CuSO <sub>4</sub> .				
67.....	6	good	semi-solid	0.100
	Months.			
68.....	6	"	"	0.100
69.....	Young lamb	"	empty	0.100 (0/3)
(c) 10 c.c. 0.5% CuSO <sub>4</sub> .	Teeth.			
70.....	6	good	semi-solid	0.100
71.....	6	"	"	0.100 (0/2)
(d) 10 c.c. 2% CuSO <sub>4</sub> .				
72.....	4	good	solid	10.90
73.....	4	"	semi-solid	(40.60)
74.....	4	"	"	0.100
75.....	4	medium	"	70.30
76.....	4	good	semi-fluid	70.30
77.....	4	"	semi-solid	0.100
78.....	4	"	"	100.0
79.....	4	"	"	0.100
80.....	4	"	"	(50.50)
81.....	4	poor	"	100.0
82.....	4	"	"	100.0 (5.4)
(e) 10 c.c. 5% CuSO <sub>4</sub> .				
83.....	4	good	solid	20.80
84.....	4	"	semi-solid	5.95
85.....	4	"	"	10.70
86.....	4	"	solid	0.100
87.....	4	"	fluid	0.100
88.....	4	"	semi-solid	0.100
89.....	4	"	semi-fluid	0.100
90.....	4	"	fluid	0.100
91.....	4	"	semi-fluid	0.100
	Months.			
92.....	12	poor	"	0.100
93.....	12	"	"	0.100
94.....	12	"	fluid	0.100
95.....	12	"	"	0.100
96.....	12	"	"	0.100
	Teeth.			
97.....	8	very poor	semi-solid	0.100
98.....	8	"	semi-fluid	0.100
99.....	8	"	"	95.5 (1/16)
(f) 3 c.c. 10% CuSO <sub>4</sub> .	Months.			
100.....	12	poor	semi-fluid	0.100
101.....	12	"	"	0.100
102.....	12	"	fluid	0.100
103.....	12	"	"	0.100
104.....	12	"	"	0.100 (0/5)
5 c.c. 10% CuSO <sub>4</sub> .	Teeth.			
105.....	8	very poor	semi-solid	0.100
106.....	8	"	"	0.100
107.....	8	"	"	0.100 (0/3)
2.5 c.c. 10% CuSO <sub>4</sub> .				
108.....	8	very poor	semi-fluid	0.100
109.....	8	"	semi-solid	100.0
110.....	8	"	solid	0.100
111.....	8	"	semi-solid	0.100
112.....	8	"	"	0.100
113.....	8	"	"	0.100
114.....	8	"	"	5.95
115.....	8	"	semi-fluid	(2.10) (1/6)

The 0·1 per cent. solution is apparently too weak. A 0·25 per cent. or 0·5 per cent. solution may give quite good results if all other factors are favourable, and this will also be the case with a 2 per cent. solution, but the results obtained with the latter were on the whole very disappointing, particularly with sheep in poor condition (Nos. 81 and 82). The 5 per cent. solution gave much more satisfactory results. The 10 per cent. solution was tested only on poor sheep and the results are very promising even with as small quantities as 2·5 c.c.

It may be difficult to draw definite conclusions from the above results since the numbers of sheep used are still small and the results may not be very convincing. However, if one considers also the results under the other headings and particularly the field experiences in dosing against *Oesophagostomum columbianum*, it becomes quite clear that there are certain other controlling factors and that, if these are favourable, a 2 per cent. solution will produce the reflex, but if these factors are unfavourable a 5 per cent. or even a 10 per cent. solution is necessary.

The chief controlling factors appear to be age and condition of the sheep and consistency of the ruminal contents. The last point has already been discussed. Young sheep can undoubtedly be more easily stimulated than older sheep, but what is much more important is the observation made in the field tests referred to, namely that sheep badly infested with a worm like *Oesophagostomum columbianum* or which for some other reason are in very poor condition, sluggish and numb, are very unsatisfactory subjects to stimulate. It stands to reason that their nervous reflexes are not as lively as are those of sheep in reasonably good condition. The article on therapy of oesophagostomiasis in this issue will show the marked difference in the results obtained after stimulation with 10 per cent. and with weaker solutions.

Unfortunately this knowledge was gained at a relatively late stage of the work and it will be noticed that the tests in other sections suffered from lack of knowledge on this point. The results all round could have been more definite if variations due to the concentration of the stimulant had not been present.

## 6. CONDITION OF SHEEP.

In order to obtain an idea of the percentage of successes that may be obtained, a test was made at a local abattoir on sheep which were being slaughtered there and, since these sheep were all in good condition and fairly excited, the results will be interesting as confirmation of the conclusions arrived at in section 5 above and in contrast to other tests reported below in this section.

The sheep slaughtered at the abattoir had been allowed to graze and drink until the previous evening and were then brought into a bare paddock. Early in the morning they were taken in lots of about 30 to small pens adjoining the slaughter hall. Here they were caught one by one and dragged into the hall, some coming in on their sides or backs, only a small minority on their feet. Just inside the door they were dosed with 10 c.c. 2 per cent.  $\text{CuSO}_4$ .

immediately followed by red lead oxide and this was done in whatever position the sheep happened to be at the time. Immediately after having been dosed, the sheep were killed and the stomachs were examined later. Of 143 sheep dosed 32 had not swallowed before they were killed. Of the remaining 111, 108 swallowed into the abomasum. The ruminal contents were fluid or semi-fluid. There were 25 persian lambs, of which 24 swallowed into the abomasum, and the other sheep were of different ages but mostly full-mouth, some being persians or cross-breeds but the majority merinos. The position in which the sheep were held at dosing did not appear to have any effect at all.

These results were certainly satisfactory and both the condition of the sheep as well as the consistency of the ruminal contents were favourable. The sheep had been grazing on young, succulent grass the previous day.

Four sheep in poor condition were driven into the post-mortem hall at Onderstepoort and an attempt was made to excite them by hitting them with a white towel and chasing them about before they were dosed, but they did not pay much attention to these efforts. They were stimulated with 10 c.c. 2 per cent.  $\text{CuSO}_4$  and then given the usual level teaspoonful of red lead oxide.

TABLE VII.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
116.....	4	poor	solid	100·0
117.....	4	"	"	90·10
118.....	4	"	semi-solid	90·10
119.....	4	"	"	0·100 (3/1)

Here the condition as well as the consistency of ruminal contents, at least in sheep 116 and 117, were unfavourable and the result was accordingly unsatisfactory.

## 7. ACIDITY OF RUMINAL CONTENTS.

It had been noticed that the ruminal contents of some sheep have a strong acid smell and that this is usually the case when the contents are on the solid side, particularly in sheep that had received a ration of grain in their food. In order to determine whether such acidity might counteract the stimulant, four sheep (*a*) were given 50 c.c. 5 per cent. sodium bicarbonate solution before the stimulant (10 c.c. 5 per cent.  $\text{CuSO}_4$ ) and indicator were administered, four others (*b*) were dosed only with water instead of bicarbonate and another four (*c*) only with stimulant and indicator. All the sheep had been given a ration of dry hay and maize for some days previously.

TABLE VIII.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
(a) 120.....	4	poor	semi-solid	0·100
121.....	4	"	"	0·100
122.....	4	"	"	(10·0)
123.....	4	"	semi-fluid	0·100 (0/;
(b) 124.....	4	"	semi-solid	0 100
125.....	4	"	"	(10 10)
126.....	4	"	semi-fluid	0·100
127.....	4	"	"	0·100 (0/;
(c) 128.....	4	"	semi-solid	(50·50)
129.....	4	"	"	100·0
130.....	4	"	semi-fluid	0·100
131.....	4	"	"	0·100 (1/;

Unfortunately the ruminal contents were not solid. The results do not indicate that there is anything in favour of the alkali, but possibly the 50 c.c. of fluid given in cases of (a) and (b) may have had a favourable effect.

## 8. ASTRINGENCY OF STIMULANT.

Since it was not clear why copper sulphate produced the stimulus, the tests under this and the next two headings were made to gain some knowledge with regard to this question. It is known that copper sulphate solutions are strongly astringent, and therefore other astringents were tested.

TABLE IX.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
(a) 10 c.c. 5% Ferric chloride,	then indicator.			
132.....	4	medium	semi-solid	100 0
133.....	4	"	"	(30·30)
134.....	2	good	fluid	0·100
135.....	2	"	"	5 95
136.....	2	poor	semi-solid	10 90
137.....	2	"	"	10·50
138.....	2	"	"	0 100 (1/5)
(b) 10 c.c. 5% Sodium alum,	then indicator.			
139.....	2	good	fluid	(50·50)
140.....	2	"	"	0·100
10 c.c. 10% Sod. alum, then indicator.				
	Months.			
141.....	12	poor	semi-fluid	100·0
142.....	12	"	"	(50 50)
143.....	12	"	"	(50·50) (1/1)
(c) 10 c.c. 10% Tannic acid,	then indicator.			
	Teeth.			
144.....	2	poor	semi-fluid	(50·50)
145.....	2	"	"	90·10
146.....	2	"	"	95·5 (2/0)

Although the results of group (a) look satisfactory the metallic-ion may have had an influence (see below, section 10); the results with alum are indefinite, while tannic acid does not give the indication that astringency is the important factor. In all cases strong solutions were used, the sheep were mostly young and the ruminal contents of favourable consistency, so that, although some of the sheep were poor, better results would have been expected with the use of copper sulphate. The results, however, are admittedly not quite convincing, and the tests should be repeated.

### 9. OTHER COPPER SALTS.

In order to make sure that it is the Cu-ion which is the active stimulant other copper salts were tested. These, it must be noted, however, all give astringent solutions.

TABLE X.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
Teeth.				
(a) 10 c.c. 5% Cu(NO <sub>3</sub> ) <sub>2</sub> , then indicator.				
147.....	2	poor	semi-fluid	2.98
148.....	2	"	"	(0.0)
149.....	2	"	"	0 100
150.....	2	"	"	0 100 (0/3)
10 c.c. 2% Cu(NO <sub>3</sub> ) <sub>2</sub> , then indicator.				
151.....	4	poor	semi-solid	0 100
152.....	6	"	semi-fluid	40 60 (0/2)
(b) 10 c.c. 5% copper acetate, then indicator.				
153.....	2	poor	semi-fluid	0 100
154.....	2	"	"	0 100 (0/2)

Sheep 152 may have to be disregarded. Otherwise it seems fairly clear that other copper salts will give the reflex.

### 10. OTHER METALS.

TABLE XI.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
Teeth.				
(a) 30 c.c. 3% AgNO <sub>3</sub> , then indicator.				
153.....	6	good	semi-solid	10.90
154.....	6	"	"	0.100
155.....	6	"	"	0.100
156.....	2	poor	"	(10.30)
157.....	2	"	"	100.0
10 c.c. 1% AgNO <sub>3</sub> , then indicator.				
158.....	2	poor	semi-solid	10.90
159.....	2	"	"	5.95
160.....	2	"	"	95.5 (2/5)

TABLE XI (Continued).

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
(b) 10 c.c. 3% CdSO <sub>4</sub> , then indicator.				
161.....	6	good	semi-solid	0 100
162.....	6	"	"	100 0
163.....	6	"	"	95 5 (2/1)
(c) 10 c.c. 3% ZnSO <sub>4</sub> , then indicator.				
164.....	6	good	semi-solid	0 100
165.....	6	"	"	10 60
166.....	6	"	"	0 100
10 c.c. 1% ZnSO <sub>4</sub> , then indicator.				
167.....	6	good	semi-solid	100 0
168.....	6	"	"	100 0
169.....	6	"	"	5 95 (2/4)
(d) 10 c.c. 3% NiCl <sub>2</sub> , then indicator.				
170.....	2	poor	semi-solid	100 0
171.....	2	"	"	70 30 (2/0)
(e) 10 c.c. 3% Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , then indicator.				
172.....	2	poor	semi-solid	100 0
173.....	2	"	"	99 1 (2/0)
(f) 10 c.c. 3% BaCl <sub>2</sub> , then indicator.				
174.....	2	poor	semi-solid	100 0
175.....	2	"	"	100 0 (2/0)
(g) 10 c.c. 3% Sodium acetate, then indicator.				
176.....	2	poor	semi-solid	(30 10)
177.....	2	"	"	100 0 (1/0)
(h) 10 c.c. 3% FeSO <sub>4</sub> , then indicator.				
178.....	6	good	semi-solid	100 0
	Months.			
179.....	9	"	"	(50 50)
180.....	9	"	"	100 0
181.....	9	"	"	30 70 (2/1)

According to the periodic table the metals Zinc and Silver are the most closely related elements to Copper, and it is interesting to see that these two have also given the best stimulus. However, no definite conclusion can be drawn from these small numbers of animals and possibly the valency of the metals may have to be taken into account—compare the results with ferric and ferrous salts (Tables IX and XI). At any rate, nothing better than the Copper salt was found, and that was the information required at the moment for practical purposes.

#### 11. DRUGS AFTER CuSO<sub>4</sub>.

It was important now to determine whether certain drugs, administered after the stimulant, might not counteract the stimulant or for some other reason cause the oesophageal groove to open..

In all the groups except (a) the substance tested was administered immediately after stimulation with 10 c.c. 5 per cent. CuSO<sub>4</sub>.



TABLE XII.

Sheep No.	Age.	Condition.	Rumen Contents.	Result.
	Teeth.			
(a) 0.5 c.c. 40% nicotine sulphate + 0.5 Gm CuSO <sub>4</sub> + 30 c.c. water + Pb <sub>3</sub> O <sub>4</sub> .	hate + 0.5	Gm CuSO <sub>4</sub> +	semi-solid	0.100
182.....	4-8	medium	"	0.100
183.....	4-8	"	"	0.100 (0/3)
814.....	4-8	"	"	
(b) 5 c.c. CCl <sub>4</sub> + 10 c.c. liquid paraffin + Pb <sub>3</sub> O <sub>4</sub> .	paraffin +	Pb <sub>3</sub> O <sub>4</sub> .	semi-solid	10.90
185.....	4-8	medium	"	30.70
186.....	4-8	"	"	10.90 (0/3)
187.....	4-8	"	"	
(c) 5 c.c. C <sub>2</sub> Cl <sub>4</sub> + 10 c.c. liquid paraffin + Pb <sub>3</sub> O <sub>4</sub> .	paraffin +	Pb <sub>3</sub> O <sub>4</sub> .	semi-solid	0.100
188.....	4-8	medium	"	0.100
189.....	4-8	"	"	100.0 (1/2)
190.....	4-8	"	"	
Many more cases have since been treated in this way with good results.				
(d) 50 c.c. 20% MgSO <sub>4</sub> + Pb <sub>3</sub> O <sub>4</sub> .				
191.....	6	good	semi-solid	100.0
192.....	6	"	"	0.100
193.....	6	"	"	0.100 (1/2)
(e) 100 c.c. 10% Jalap + Pb <sub>3</sub> O <sub>4</sub> .				
194.....	6	good	semi-fluid	0.100
195.....	6	"	"	0.100
196.....	6	"	semi-solid	0.100 (0/3)
(f) ½ teaspoon calomel + ½ teaspoon Pb <sub>3</sub> O <sub>4</sub> .	teaspoon Pb <sub>3</sub> O <sub>4</sub> .			
197.....	6	good	semi-solid	0.100
198.....	6	"	"	0.100 (0/2)
(g) 50 c.c. ol. lini + Pb <sub>3</sub> O <sub>4</sub> .				
199.....	6	good	semi-solid	0.100
200.....	Months.			
	6	"	"	0.100 (0/2)
	Teeth.			
(h) teaspoonful As <sub>2</sub> S <sub>3</sub> powder.				
201.....	6	good	semi-solid	90.10
202.....	6	"	"	10.90 (1/1)
(i) 30 c.c. 3% Lugol's iodine + Pb <sub>3</sub> O <sub>4</sub> .	+ Pb <sub>3</sub> O <sub>4</sub> .			
203.....	6	good	semi-solid	10.90
204.....	6	"	"	0.100 (0/2)
(j) 30 c.c. 1% Tarter emetic + Pb <sub>3</sub> O <sub>4</sub> .	+ Pb <sub>3</sub> O <sub>4</sub> .			
205.....	6	good	semi-solid	0.100
206.....	6	"	"	0.100 (0/2)
(k) 30 c.c. 1% Gentian violet + Pb <sub>3</sub> O <sub>4</sub> .	+ Pb <sub>3</sub> O <sub>4</sub> .			
207.....	6	good	semi-solid	0.100
208.....	6	"	"	0.100 (0/2)
(l) 30 c.c. water at 40° C. + Pb <sub>3</sub> O <sub>4</sub> .	Pb <sub>3</sub> O <sub>4</sub> .			
209.....	6	good	semi-solid	0.100
210.....	6	"	"	10.90 (0/2)
30 c.c. water at 50° C. + Pb <sub>3</sub> O <sub>4</sub> .	Pb <sub>3</sub> O <sub>4</sub> .			
211.....	6	good	semi-solid	0.100
212.....	6	"	"	0.100 (0/2)
(m) 3 hard tablets each 9 mm. in diameter, 4.5 mm. thick in centre and tapering towards the edges. Tablets not shown in result were not found in sheep, probably spat out.	in diameter, 4.5 mm. thick in centre and tapering towards the edges. Tablets not shown in result were not found in sheep, probably spat out.			
213.....	6	good	semi-solid	0.2
214.....	6	"	"	0.3
215.....	6	"	"	0.2
216.....	6	"	"	1.0
217.....	6	"	"	0.3 (1/4)
(n) 1 gelatine capsule 100 mg.. filled with Pb <sub>3</sub> O <sub>4</sub> .	filled with Pb <sub>3</sub> O <sub>4</sub> .			
218.....	6	good	semi-solid	1.0
219.....	6	"	"	1.0
220.....	6	"	"	1.0 (3/0)

The results show that the oesophageal groove is firmly closed and that ordinary drugs will not cause it to open. Even small tablets are frequently swallowed through—a point which should be further investigated and which may be of great value in therapeutic work—but that large bodies such as a gelatine capsule measuring 25 by 8 mm. will still pass to the rumen.

## 12. RATE OF PASSAGE OF DRUGS THROUGH ALIMENTARY TRACT.

It was next attempted to find at what rate drugs administered into the abomasum would pass through the intestine since this point is of some importance in therapy against worms in the colon. The work done up to the present is not yet very informative, but the following results may be given.

Eight sheep were dosed with 10 c.c. 5 per cent.  $\text{CuSO}_4$  followed by a teaspoonful of red lead oxide.

(a) Killed after 1 hour:—

Sheep 221, 4t., poor, powder in abomasum and duodenum.

Sheep 222, 4t., poor, powder in abomasum only.

(b) Killed after 3 hours:—

Sheep 223, 4t., poor, powder in forestomachs.

Sheep 224, 4t., poor, powder in abomasum and down to 18 feet anterior to ileo-caecal valve.

(c) Killed after 5 hours:—

Sheep 225, 4t., poor, powder in abomasum and down to ileo-caecal valve.

Sheep 226, 4t., poor, powder in abomasum and down to beginning of ansa spiralis of colon.

(d) Faeces analysed for lead at intervals. Dosed 11.0 a.m.

Sheep 227, 4t., poor, first traces in faeces after 5 hrs. (4 p.m.), strong after 22 hrs. (9.0 a.m. next day).

Sheep 228, 4t., poor, nothing in faeces 4 p.m., strong after 22 hrs.

Another six sheep were dosed as follows: 229-231 given 10 c.c. 1 per cent.  $\text{CuSO}_4$  followed by a teaspoonful copper arsenate, 232-234 given 10 c.c. 5 per cent.  $\text{CuSO}_4$  followed by the same drug. The sheep were dosed at 4.30 p.m., 11.10.34, and the presence of copper was determined in the faeces at intervals. In the record of results — indicates absence of copper, (+) a trace, + small quantity, ++ large quantity, 0 no faeces passed.

Sheep.	12.10.34. 9.0 a.m.	10.30 a.m.	2 p.m.	4 p.m.	13.10.34. 9.0 a.m.
229.....	(+)	0	0	+	+
230.....	—	0	0	0	0
231.....	—	+	+	0	+
232.....	(+)	0	++	0	++
233.....	—	(+)	0	++	++
234.....	—	(+)	0	0	+

It is, of course, not known which of these sheep had swallowed the drug into the abomasum.

The results appear to indicate that the drug passes out of the abomasum in small quantities over a period of several hours, that the first amount may pass the ileo-caecal valve about 5 hours after treatment and that it remains in the colon for several hours, usually not being passed in the faeces before 15 hours have elapsed since the time of treatment, the bulk being passed from about the 22nd hour. It must, however, be expected that marked variations may occur, depending on the nature of the drug itself, the food eaten by the sheep and the general digestive conditions in the alimentary tract at the time of treatment, for instance the degree of acidity of the abomasal contents. In therapeutic work on *Oesophagostomum columbianum*, in which the results have been observed on several hundred sheep, it was found that the first worms may be passed from about 15 hours after treatment, while the majority are passed after that time and sometimes for a period up to five days, but this does not necessarily mean that the drug remains in the colon all that time.

### 13. THE DANGER OF ADMINISTERING DRUGS INTO THE ABOMASUM.

This article would not be complete without a warning against the dangers involved in administering drugs into the abomasum. The mucosa of this organ is remarkably tender, apparently much more so than the gastric mucosa of non-ruminants, and it is consequently very easily corroded by drugs which come in contact with it in a concentrated form. In therapeutic tests it was found that this danger is not by any means negligible. For instance, sheep dosed with 1 gm. sodium fluosilicate after copper sulphate all died and showed a localised necrosis surrounded by an inflamed area in the abomasal mucosa. Also some farmers who had heard about the method of administration and administered various remedies for *Haemonchus contortus* in this way, lost considerable numbers of sheep through remedies which they had previously used successfully.

### SUMMARY AND CONCLUSIONS.

(1) A solution of copper sulphate stimulates reflex closure of the oesophageal groove in sheep so that drugs subsequently administered will pass into the abomasum.

(2) Preliminary starvation has no favourable influence, unless the sheep are starved for such a long period that the ruminal contents become fluid, in which case the latter condition is favourable. Starvation is, however, contra-indicated for reasons which are discussed.

(3) The method of administration and the position in which the animal is held are of no importance.

(4) The reflex is established immediately after the stimulant touches the mucosa of the pharynx.

(5) The groove remains closed for 15 seconds, sometimes longer, after stimulation.

(6) Other conditions being favourable, the stimulus can definitely be produced by a 0.25 per cent. copper sulphate solution. Unfavourable factors are advanced age, poor condition and dryness of ruminal contents. In it shown that a 10 per cent. solution is necessary to overcome the counteracting effects of these adverse factors.

(7) It has not been definitely proved whether acidity of the ruminal contents is an unfavourable condition, but there are indications that this is not the case.

(8) Copper salts other than the sulphate produce the reflex, but it has not been definitely proved that the stimulus may not be due to astringency of the stimulant.

(9) There are indications that the related metals zinc and silver may also produce the reflex.

(10) Various drugs administered after the stimulant did not reverse the reflex. Small pills may be swallowed into the abomasum after stimulation but larger objects (small capsules) are not.

(11) Preliminary tests indicate that a drug administered into the abomasum may reach the colon in about five hours and that it may be passed in the faeces from about the 15th hour.

(12) Since the abomasal musosa is very tender, care must be exercised in administering drugs in the way indicated.

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## **Studies on the Alimentary Tract of Sheep in South Africa.**

### **III. The Influence of Bowel Anastomosis on the General Nutritional State of Merino Sheep.**

By

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#### **INTRODUCTION.**

IN his "Mechanics of the Digestive Tract", Alvarez aptly quotes Billings to the effect that "a good reliable sett ov bowels iz wurth more tu a man than enny quantity of brains". Loss of moderate portions of this tract leaves the individual with little disturbance. Ileo-transversostomy is an operation of common occurrence and on the whole well-borne in man. Resections of small gut pass by unnoticed. When however extensive portions of small intestine are sacrificed a margin of safety is approached beyond which health and vigour may become severely impaired. Observations of this type are equally numerous and varying, varying for the simple reason that all measurement of bowel in vivo is relative. The standard anatomical length of 22 feet of small gut in the human can only be adhered to as a post-mortem fact. Attached to its fan-shaped mesenterium, and subject to the play of varying degrees of tonus at operation, it is possible to estimate the same length of bowel at the most divergent figures. Thus the cadaver length of 5.6 metres of small intestine is reduced to a total length of 2.5-2.75 metres from mouth to anus by swallowing of tubes in the experiments of Schembra and v. d. Reis. Moreover the normal divergence in bowel length in different individuals is such that the effects of extensive resection can rarely be gauged by measuring the excised length of bowel, and therefore only careful consideration of the remaining length of functioning tract can act as a guide to the re-establishment of equilibrium.

It would appear that loss of anything above half the length of small bowel can lead to metabolic disturbances. Numerous such cases are reported after resection of 2 meters of small gut (strangulations and lesions post abortum occurring frequently). Even loss of shorter stretches has led to disturbance, the subsequent autopsy revealing that the patient had originally had a much shorter intestinal tract than the classical length. In general the vast majority of extensive resections appertain to small gut with subsequent anastomosis of upper to lower small intestine.

The occasion for intestinal exclusion necessitating an anastomosis between jejunum and colon is doubtless rare, and as such the problem of the permissibility of joining a minimum functional length of the small intestine to a given part of the colon has received scant attention. The experience of such a case stimulated our interest in this question. Briefly, a man aged 58, had recently before admission into hospital recovered under conservative treatment from a subacute attack of ileus. The abdomen was still moderately distended, and there was still evidence of bowel-distension. Barium enemas proved negative. Oral administration revealed that a blind pouch became filled from the upper coils of the small bowel, and this in conjunction with a scar in the left hypochondrium from draining some cystic cavity 15 years previous, seemed to establish the diagnosis of a pancreatic cyst which might by adhesions be causing an incomplete obstruction. Laparotomy was agreed upon. At the operation one found practically all the distal  $\frac{2}{3}$  of the small intestine embedded in one extensive mass of adhesions, superimposed upon countless cysts of the appearance of extremely thin walled dermoid cysts situated throughout the mesenterium of the small gut, and binding down the whole conglomerate mass to the caecum and ascending colon. At one spot a definite kink in the whorls of intestine had caused obstruction, as was evidenced by the dilatation of gut extending three feet proximally. Three courses were open to pursue:—

1. A radical resection of all involved gut and proximal colon in the presence of partial ileus would have meant certain death;
2. separation of such adhesions as were causing the bowel obstruction was a rational, safe line to follow, but uncertain in its effect and the duration thereof;
3. an anastomosis between the free proximal small intestine to the colon could not be applied in the form of a jejuno-caecostomy, the whole mass preventing such approximation, and would of necessity have had to become a jejuno-transversostomy.

In view of the extreme uncertainty of such a procedure, recourse had to be taken to the second choice, that of liberating the strictured gut. A cyst excised intact for examination revealed a laminated membrane of echinococcal cyst with sterile debris as contents.

In view of the above case it was decided to perform a series of investigations upon animals to attempt to ascertain whether the proximal half of the colon was of essential value in cases with a minimal residue of small intestine, in casu, jejunum. There seemed reason to believe that the function of the proximal colon is of sufficient importance to provide different results in jejuno-caecostomy as compared to jejuno-transversostomy.

The hind-gut, in man and in most animals, has repeatedly been shown to act mainly as a faecal reservoir with comparatively little absorptive or excretory function to perform. In man the hind-gut commences at the junction of the middle and distal third of the transverse colon. That part of the large intestine which would be

excluded in a jejuno-transversostomy forms the distal part of the mid-gut. It has far greater significance than the distal colon or hind-gut. Such is already evidenced by its greater diameter, the faecal end of the colon having a diameter of 6 c.m. as compared to 2.5 c.m. at the distal sigmoid. The greater volume corresponds to the pre-eminent absorptive function of the proximal colon. Intestinal contents are discharged into the caecum in fluid state, and in the distal third of the transverse colon, that is on entering the hind-gut, are transformed into inspissated faecal balls. Nature effects this transformation over a short course by various ingenious manoeuvres of this portion of the gut, which has lately been intensively elucidated by X-ray cinematography (Wildegans). Chief of these is that peculiar movement of antiperistalsis, almost exclusively confined to this portion of the alimentary canal, and only questionably present in the sigmoid colon under other than pathological conditions. Antiperistalsis, moreover, is responsible for faecal tumor formation in the proximal colon in cases of intestinal exclusion, a subject upon which Tonnis offers considerable elucidation.

The extreme difference in the size and shape of the proximal colon in carnivorous and herbivorous animals is sufficient proof of its function of digesting cellulose products in addition to its condenser action. Man, the omnivorous forms an intermediate, with a haustrated colonic structure resembling the herbivorous type. Its comparative shortness in man on the other hand shows that it is designed for handling only small amounts of cellulose. Primitive man was a hunter and fisherman thousands of years before he learned to till the soil (Alvarez).

The question therefore arises: can the proximal colon cope with semi-digested intestinal contents from the jejunum in the sense of its normal function, and thus ameliorate the extreme digestive deficit of a jejuno-transversostomy? And, alternatively, does the proximal colon, in virtue of its antiperistaltic function possibly still function by retrograde filling from a jejuno-transversostomy in such cases of intestinal exclusion? Do such experiments shed any further light on faecal tumor formation in the proximal colon?

The choice of animal would fall on monkeys, which, however, could not be obtained in sufficient number and similarity for such experiments. Carnivora with their extremely short colon would not justify any comparison to man, although the intestines of the dog are most favourable for manipulation and measurement. Pigs would suit the purpose but for the difficulty in handling them. Rabbits were tried as a first attempt at herbivora, but it appeared that the mortality from shock within 24 hours (without technical deficiencies at the anastomosis) proved too great. It was therefore decided to select sheep.

The colon in man corresponds mostly to that of the herbivorous animal, which is blessed with an extensive large bowel for the digestion of its cellulose nourishment. One felt too, that if there was no appreciable divergence between jejuno-caecostomy and jejuno-colostomy in herbivora one would be justified in concluding that the divergence should be relatively equally small in man. Unfortunately



the measurement of small intestine is exceptionally difficult in the proximal length of jejunum in the sheep, where the mesenterium is so short, and the bowel so deepseated that the utmost care is required to avoid mesenterial haemorrhage. As a point corresponding to the distal part of the transverse colon in man, one selected the mid-point of the ansa spiralis in the sheep, a point easily recognisable and constant, and a point moreover where the ingesta have become completely "balled". Absorption must therefore have been practically completely effected before reaching this point.

#### OPERATIVE PROCEDURE.

As experimental animals were selected 4-tooth Merino hamels in good condition and of clinically healthy appearance. All animals had been kept under laboratory conditions for some months prior to being placed in experiment. The ration both before and after operation consisted of good quality veld hay, green barley or green lucerne and a small daily allowance of yellow crushed maize, with drinking water *ad lib*. Records were kept of the appetite shown by animals at different times, although the actual daily quantities consumed were not recorded. At first all animals were weighed twice weekly although later on this was changed to one weighing weekly. Furthermore faeces were collected from all animals twice weekly in linen bags strapped round the anus, and the amount, consistence and other characteristics described. For the rest, the animals were kept under constant clinical observation and post-mortem examination carried out on all animals lost, special attention being paid to the measurements of the different parts of intestine.

After 48 hours total starvation and prior to operation, the abdomen of the animal was shorn, shaved and washed and disinfected. Choral hydrate 60 to 80 c.c. 10 per cent. intravenously was used throughout as a general anaesthetic, the results on the whole being very satisfactory. All operations were performed under aseptic precautions. The first step was a paramedian laparotomy extending from the level of the umbilicus 5 to 6 inches backwards. Following this, the caecum was partly withdrawn and the ileo caecal junction and lower ileum made out. Starting either from the ileo caecal opening or from the duodenum, certain lengths of small intestine were measured off in approximately 6-inch loops caught up progressively between the thumb and forefinger of the two hands. This measurement as can be understood was at best only relative, seeing that the intestines are extremely elastic and festooned on the mesentery. Next the bowel was caught up in a rubber protected intestinal clamp over a length of  $\pm 6$  inches at a level previously decided upon. Depending on the anastomosis to be performed, a second clamp was placed either on the upper blind end of the caecum for a jejuno-caecostomy or on to the ansa spiralis, i.e. at the junction of upper and middle third of the colon for a jejuno-colostomy.

Previous measurements of the intestines of 4-tooth sheep gave values of 55 to 60 feet for the whole length of small intestine and 13 feet for the large intestine, taken immediately after slaughtering of the animals.

After drawing up the clamps closely parallel to each other, a side-to-side anastomosis was made with an opening of approximately 2 to 3 c.ms. between the two loops of intestines which was united by a single layer of a continuous cat gut suture. Following this, the small intestine was doubly ligated just distal to the anastomosis and severed between the ligatures, the two stumps being buried by purse-string sutures. In this manner a direct passage could be established between any level of the small intestine and any part of the large intestine, the portion of small intestine distal to the anastomosis being left in situ with its upper end blind.

The abdominal wound was closed with the usual three layers of sutures and collodion iodoform dressing applied to the skin.

### EXPERIMENTAL FINDINGS.

Altogether 18 sheep were used in these experiments, being divided into two main groups of 8 each and one small group consisting of only 2 sheep.

#### *Group 1.*

In this group, comprising 8 sheep, the proximal 30 ft. of the small intestine as measured at the time of the operation, was anastomosed on to the tip of the caecum, the rest of the small intestine being left intact except for a closure and invagination of its upper stump. In the following table details are given concerning the bowel lengths as measured at autopsy, the ratio of anastomosed small intestine to the distal closed portion, and also the time of death after operation.

TABLE 1.

Sheep No.	Proximal small intestine anastomosed.	Distal small intestine.	Ratio of prox. to distal small intestine.	Large intestine.	Time of death.
41014	24 ft.	28 ft.	1 to 1.17	—	7 weeks.
40596	25 ft.	36 ft.	1 to 1.44	13½ ft.	8½ weeks.
40697	14 ft.	25 ft.	1 to 1.8	13 ft.	10 weeks.
40585	21 ft.	29 ft.	1 to 1.4	11½ ft.	10 weeks.
40893	29 ft.	33 ft.	1 to 1.1	13 ft.	19 weeks.
41051	23 ft.	41 ft.	1 to 1.8	—	1 day (shock).
40846	30 ft as measured at operation	—	—	—	Alive after 31 weeks.
38246	25 ft. as measured at operation anastomosed on to ileum 3 ins. above ilco-caecal opening	—	—	—	Alive after 44 weeks.

As will be seen from the above table, the total length of small intestines in the different sheep showed considerable variation although the length of large intestine was fairly uniform throughout. Furthermore in all the sheep which died, the length of small intestine anastomosed was shorter than that excluded, i.e. in every case actually less than half of the whole small intestine was kept in full function. In some cases this was only 4 ft. less than the distal portion excluded while in others it was considerable and measured as much as 11 ft. However, by anastomosing on to the tip of the caecum the whole large intestine was kept in function. Of the 8 animals only 2 are still alive, 5 of the others having died at intervals ranging from 7 to 19 weeks after operation, while 1 died from shock 24 hours after operation.

*Clinical Symptoms.*—The only symptoms of interest during the experimental period were those in connection with the general nutritional condition including the body weights and the amount and nature of the faeces passed.

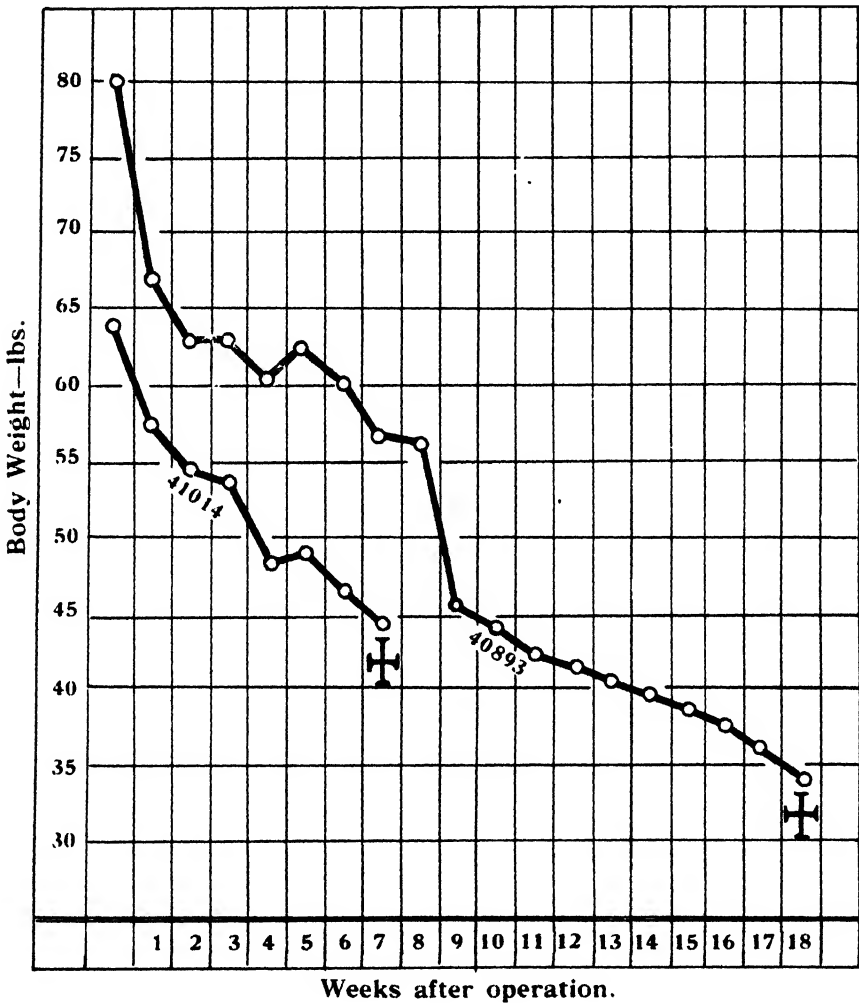
*Faeces.*—All animals, except one, stood the operation very satisfactorily, and usually commenced feeding on the second or third day. In every case, however, a severe watery diarrhoea made its appearance within 5 days after operation. A similar attack of severe diarrhoea was repeatedly noticed to affect some of the animals at intervals of one to three weeks, the consistence of the faeces being very fluid, clear or only slightly turbid, brownish, and at worst only mildly catarrhal. With the disappearance of the diarrhoea, usually after 2 to 3 days, the consistence of the faeces rapidly increased to that of a fairly thin to thick pultaceous mass. At times there were even signs of the normal pellet formation. The colour remained fairly constant ranging from a light yellowish green to the dark green of normal sheep's faeces. The coarseness of the faecal matter showed little change and small pieces of undigested maize were at times observed in the excreta.

*Body Weight.*—In each of the 5 animals that died, there was a progressive decrease in the body weight up to the time of death as will be seen from graph 1, taken from the two sheep that died in 7 weeks and 19 weeks respectively. In every case there was progressive emaciation, and weakness, the animal frequently appearing huddled up and with the back arched. Towards the end, profound weakness was followed by prostration with decubitus. Animals found in this state were slaughtered for post-mortem examination.

In the two animals that survived, a sharp initial decrease in the body weight was followed by a gradually progressive recovery. Thus as shown in graph 2 one sheep has practically reached its original body weight after 31 weeks, while the other after 43 weeks shows considerable gain on its weight prior to the operation. Both animals appear lively, they feed well and the faeces passed is normally formed.

GRAPH 1.—Sheep 40893 and 41014.

Proximal part of small intestine anastomosed on to tip of Caecum. Both dead.

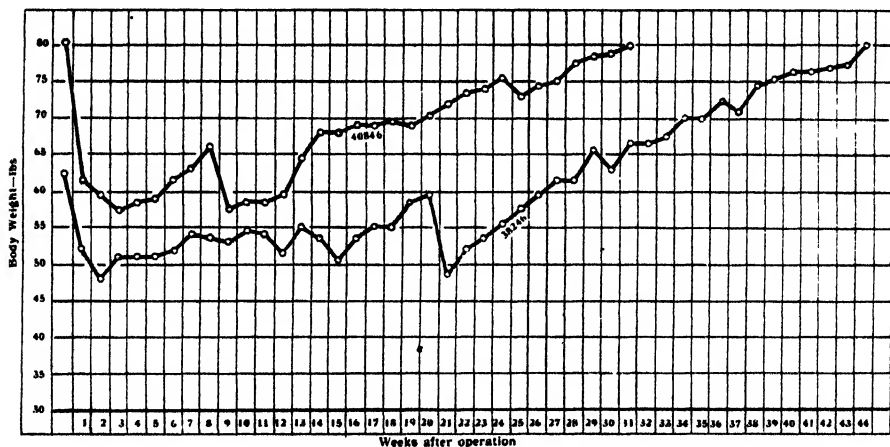


*Post-mortem Findings.*—In all five carcasses examined, the pathological changes noted were very uniform, varying slightly in degree only. Thus in every case there was definite cachexia, general atrophy of skeletal muscle and fatty tissues, gelatinous infiltration of subcutaneous tissue, varying degrees of atrophy of the internal organs, especially the liver and kidneys, accompanied by brown pigmentation, also general pallor of the carcass. The forestomachs and abomasum appeared small and in some cases badly filled and with signs of atony of the muscular wall. The proximal (anastomosed) portion of the small intestines in every case appeared to have suffered little or no change, being of the usual thickness and colour and containing apparently normal ingesta. The distal (excluded) part of

the small intestines, however, was materially altered in every sheep, being pale, markedly atrophic and threadlike. The lumen of the bowel was very small and contained only traces of a thick, creamy inspissated secretion. No food residues whatever were found in this part, neither did any retrograde filling take place from the caecum through the ileo-caecal opening. In every case the anastomosis was found widely patent, easily admitting the index finger. It appeared well healed and without signs of any leakage. The large intestine appeared well filled, the ingesta showing little change from the normal. No morphological change was detected in the walls of the large gut. In two cases there was evidence of skeletal change, e.g. rib fractures followed by poor callus formation. In these cases the ribs were found to be rather easily broken between the fingers. Samples of the different bones were collected for further examination.

GRAPH 2.—Sheep 40846 and 38246.

Proximal part of small intestine anastomosed on to tip of Caecum. Both alive.



### Group II.

In this group, again consisting of 8 animals, the proximal half of the small intestine, measured off as being approximately 30 ft., was anastomosed on to the middle of the ansa spiralis, a point found to correspond to the junction of the proximal and middle thirds of the large intestine. It should be indicated that at this level the faecal pellets are already fairly well formed and of firm consistence proving that water absorption and consequently the dehydration of the faecal mass is largely conducted in the upper third of the large intestine. Thus in this series approximately the lower half of the small intestine as well as the upper third of the large intestine was excluded by the anastomosis.

The following table indicates the different bowel lengths as found at autopsy, also the time of death after operation.

TABLE II.

Sheep No.	Prox. small intestine (anastomosed).	Distal small intestine (excluded).	Prox. large intestine (excluded).	Distal large intestine (anastomosed.)	Time of death.
40511	19 ft.	30 ft.	4½ ft.	10 ft.	3 days (obstruction).
40880	25 ft.	—	—	—	10 days (diarrhoea).
39466	18 ft.	30 ft.	4 ft.	9 ft.	11 weeks.
32865	28½ ft.	23½ ft.	5 ft.	8½ ft.	12 weeks.
39412	22 ft.	23 ft.	5½ ft.	8 ft.	21½ weeks.
40902	24½ ft.	30½ ft.	4½ ft.	8½ ft.	21½ weeks.
41089	23½ ft.	20 ft.	4½ ft.	8 ft.	30½ weeks.
38632	25 ft. as measured at operation	—	± 4 ft.	± 8½ ft.	Alive after 52 weeks.

As shown in Table II only 1 out of the 8 animals still survives. Two animals were lost within a few days after operation, one as the result of acute ileus following oedematous closure of the anastomosis wound in the intestines. The other sheep died as the result of a severe and obstinate diarrhoea appearing on the second day after the operation and lasting up to the time of death. None of the sedatives and astringents administered effectively stopped the condition. As in the previous group, the total length of small intestines in the different animals showed considerable variation, although the large intestine was of closely uniform length in all the sheep. Except in the case of two animals, the length of small intestine excluded was again greater than that which was anastomosed. With regard to the large intestine, the upper 4 to 5 feet was regularly excluded in every animal while approximately twice this length was retained through the anastomosis. Five animals succumbed over a period ranging from 11 to 30½ weeks after being operated. One sheep is still alive after 12 months.

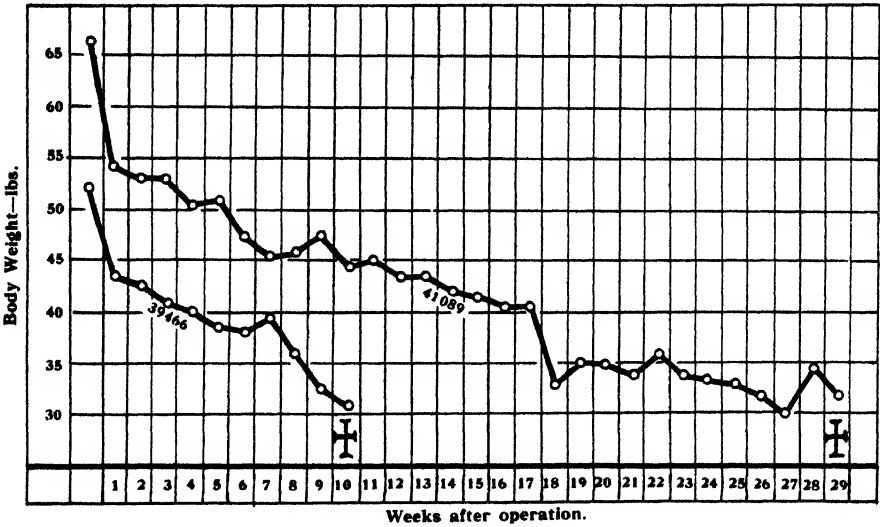
*Clinical Symptoms.*—The symptoms shown by all animals in this group, except for the two acute cases, were very closely similar to those seen in the first group. In every case, however, the diarrhoea was more persistent, although the degree of wateriness of the faeces varied from time to time. Normally formed solid faecal pellets were never collected from any of these sheep. Even the one animal which is still alive after 12 months, has shown a diarrhoea during the whole period, at times so severe as to provoke extreme exhaustion verging on prostration.

The weight curves too are very similar to those shown by the first group. Graph No. 3 is that of the sheep which died after 11 weeks and 30½ weeks respectively, while the animal still surviving is represented on graph No. 4. This latter animal has shown remarkable tenacity in spite of the severe and repeated attacks of diarrhoea, which are clearly represented on the graph by the numerous sharp decreases in its body weight. Whereas none of the other animals appear to have made any serious attempt at accommodating themselves to the new conditions of digestion, this one sheep has not only succeeded in adapting itself to existing conditions but has actually gained 14 lb. on its original weight as registered at the time of operation.

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GRAPH 3.—Sheep 41089 and 39466.

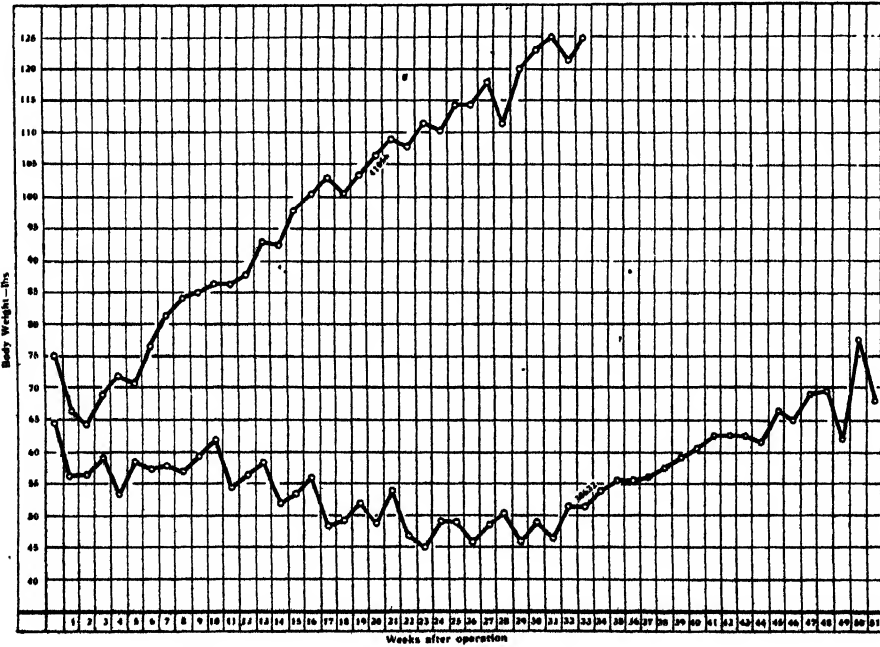
Proximal part of small intestine anastomosed onto ansa spiralis. Both dead.



GRAPH 4.

Sheep 41056. Lower ileum anastomosed onto ansa spiralis

Sheep 38632. Proximal part of small intestine anastomosed onto ansa spiralis.



*Post-mortem Findings.*—On the whole the pathological lesions are in accord with those described for the first group. The degree of cachexia and atrophy was particularly severe in those animals dying after the longest interval. The skeleton appeared small and stunted. The internal organs especially the liver, kidneys and spleen showed well marked atrophic changes. Thus the liver of sheep No. 41089 weighed 180 grams compared with the liver of a healthy sheep of the same age weighing 550 grams. (See photograph No. 1.) The distal part of the small intestines excluded from the anastomosis was again found to be pale, atrophied and empty except for small amounts of inspissated secretion. The caecum and upper colon proximal to the anastomosis in every case contained a thick pasty, greyish inspissated material probably composed of various substances such as mucins, cholesterol and mineral salts. Further analytical work is being conducted on these materials. The mucous membrane of this part showed no change from the normal. In none of the animals examined at post-mortem were there any signs of retrograde filling of the colon cranial to the anastomosis, whereas the rest of the colon distal to the anastomosis in each case contained soft pultaceous faecal masses. Although all these animals frequently showed diarrhoea, no signs of enteritis were to be noted at post-mortem.



PHOTOGRAPH No. 1.

*Left.*—Liver and kidney of sheep 41089 showing severe atrophy after loss of 20 ft. small intestine and 4½ ft. large intestine, 6½ months previously.

*Right.*—Liver and kidney of normal sheep of same age.

### *Group III.*

In order to ascertain what rôle the ileocaecal sphincter played in controlling the passage of ingesta from the small intestine to the caecum, the lower ileum in one sheep ( $\pm 6$  inches above the sphincter)



was anastomosed on to the tip of the caecum, i.e. a widely open passage was established between the small intestine and the large intestine the ends of the intestine distal to the anastomosis being closed. Regular examination of the faeces showed that this animal never suffered even a single attack of diarrhoea while the consistency of the excreta remained normal over a period of 10 months. The weight curve has also gradually risen, the animal having more than doubled its weight prior to operation. In the second sheep of this group, the lower ileum was anastomosed on to the ansa spiralis thus excluding not only the ileo caecal sphincter but also the caecum and upper third of the colon. As will be seen from its weight curve (graph No. 4) a sharp decrease immediately after the operation, was followed by a steady and progressive increase until after a period of 7 months the animal had practically doubled its original weight. Throughout the whole period the faeces were soft, thickly pulpaecous and at times appeared as partly formed pellets lumped together, i.e. the degree of dehydration has constantly remained somewhat below the normal.

### DISCUSSION.

Experiments were carried out on Merino sheep (4 tooth old), with the object of ascertaining firstly, the effect of anastomosing the upper half of the small intestines on to the caecum and secondly, by anastomosing the same portions of small intestines on to the ansa spiralis, corresponding to the junction of upper and middle thirds of the large intestines. For this purpose two main groups were formed of eight sheep each. On the whole the operation was well stood, only three sheep being lost within the first week, one from shock, another from ileus through oedematous swelling and closure of the anastomosis and a third from acute and persistent diarrhoea. Of the remaining thirteen sheep, 10 died over a period of 12 months. The main difficulty encountered in the operation was in connection with the proper measurement of the lengths of intestines especially of the small intestine, due to the varying degrees of tonus and also to the festooned attachment of the intestine on to the mesentery. In all cases the object was to preserve approximately half the small intestine through the anastomosis and to exclude the other (distal) half. However, post-mortem examination and measurement of the intestines of the 10 sheep revealed the fact that in the majority of cases considerably more than half of the small intestine was excluded from the functional tract, a fact which definitely contributed to the high mortality. However, as all the carcasses showed varying degrees of cachectic and atrophic changes in which the intestines were also involved, the measurement of the latter at post-mortem may to some extent have been influenced by these processes. Thus whereas the functioning portion of the intestine appeared normal, the excluded part was definitely atrophied, pale, and atonic, which to some extent might have accounted for the greater lengths obtained as compared with those of the functioning portions. No suitable method has been devised for a more accurate measurement of intestinal length, although this point is being investigated in connection with a further series of experiments about to be undertaken.

All the operated animals lost weight very rapidly in spite of their good appetites. The 10 animals which died, never even seemed to make an attempt to adapt themselves to the shorter intestinal lengths and thus inhibit the progressive body wastage. Of the three surviving animals a preliminary period of severe wasting was followed by a second phase during which gradual adaptation took place. The animals not only maintained their weight during this period but were actually able to show a steady gain under the new conditions of digestion and absorption. Unfortunately no detailed study of the metabolism of these animals was possible at the time of the experiment.

Of the two groups, the animals in the first group, in which only the lower half of the small intestine was excluded, showed no significant advantage over the second group in which the proximal part of the large intestine was in addition also excluded from the anastomosed tract. In no instance was there retrograde filling of the lower half of the small intestine from the caecum, neither did the caecum and proximae part of the colon become filled where the anastomosis was made on to the *ansa spiralis*. This fact indicates that retroperistalsis is extremely feeble or perhaps completely absent in the upper third of the large intestine of the sheep. The large amount of inspissated non-faecal material regularly found in the caecum and upper colon indicates that this is an area of active excretion and secretion. Complete analyses of these materials are at present being carried out. Although the caecum and upper colon must be regarded as of great importance in the absorption of water, the lower levels of the colon are to some extent capable of compensating for the loss of the caecum and upper colon. This however is largely a matter of individuality as some animals suffer heavy loss of water through a persistent diarrhoea while others minimise this loss by passing less watery faeces. The diarrhoea so often noticed in the animals, was however not related to any form of colitis as no pathological lesions were found in the large colon post-mortem. In two animals operated upon, the loss of the ileocaecal sphincter only or the ileocaecal sphincter together with the caecum and upper colon, led to no untoward effects whatsoever.

#### SUMMARY.

1. Exclusion of the lower half of the small intestines alone (see graphs 1 and 2), or in addition exclusion of the caecum and upper colon through bowel anastomosis causes progressive atrophy, extreme cachexia and even death in Merino sheep (see graphs 3 and 4).

2. No retrograde filling takes place of any of the portions of intestine excluded, hence the absorptive surface is decreased by the length so excluded from anastomosis.

3. There is considerable disturbance in the water metabolism of such animals, the degree of adaptation and compensation depending in some measure on the individual characteristics of the animal itself.

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## Section VII.

# Animal Husbandry.

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## **A Freak Merino Fleece.**

By V. BOSMAN, M.Sc., Sheep and Wool Research Officer,  
Onderstepoort.

A FREAK fleece of a merino ewe from the flocks of the Grootfontein School of Agriculture\* is here described. The sheep was peculiar in that it had a distinctive band, about three inches wide, of coarse long wool growing transversely across the back, stretching over the sides towards the belly region. (Fig. 1.) A cross-section through the freak region (Fig. 2), showed that the wool projected in length beyond the normal fleece. In addition, the freak wool was considerably coarser than the normal fleece and had large definite crimps. In fineness, it measured  $20.2\mu$  or a 64's quality number. The normal wool of the animal anteriorly and posteriorly to the freak wool measured  $17.7\mu$  or an 80's quality number. The average straight length of the fibres in the freak region was 19.6 cms. while the adjacent normal wool was 15.4 cms. As regards the number of follicles growing per unit area of skin, the freak region produced 1,600 per square cm. while the normal growth on adjacent regions was 4,400 per square cm. anterior to the freak region, and 4,100 per square cm. posterior to this. When wool production, per unit area of skin was compared, the freak region produced .50 grams of clean dry wool per 4 square cm. of skin, while the adjoining regions gave anteriorly .79 grams and posteriorly .75 grams of clean dry wool per 4 sq. cm. of skin.

\* The author is much indebted to Mr. G. S. Maré, Principal Sheep and Angora Officer of the Union, for the material.

A FREAK MERINO FLEECE.

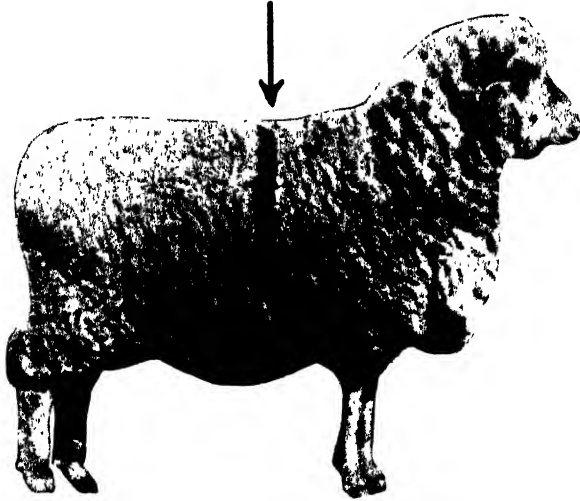


Figure 1.—The Merino Sheep with the freak region (indicated by the arrow) growing as a distinctive band across the back and sides of the animal.

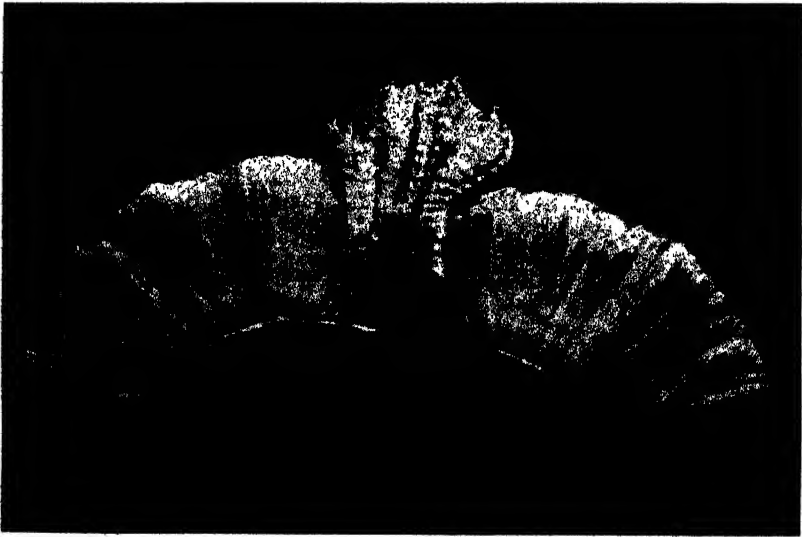


Figure 2.—A cross-section through the freak region, showing distinctive differences between the freak wool and the normal wool.

## **Wool Studies.**

### **I. The Variation and Interdependence of Merino Fleece and Fibre Characteristics.**

By A. P. MALAN, M.Sc., Statistician, C. M. VAN WYK, M.Sc.,  
and M. L. BOTHA, B.Sc(Agric.), Wool Research Officers,  
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#### **I. INTRODUCTION.**

THE sheep used at Onderstepoort for experimental purposes are as a rule obtained from other centres, where conditions are more suited to breeding. Soon after their arrival here, marked changes are to be observed in the wool produced by the animals. It is well known that changes in environmental conditions may produce considerable changes in the wool, and the individual contributions of changes in such factors as meteorological and nutritional conditions have been studied to a certain extent.

As an example may be quoted the work done at the Grootfontein School of Agriculture, from where many of the sheep used at Onderstepoort are obtained. It has been demonstrated at that Institution (Maré and Bosman, 1934) that underfeeding produces lighter fleeces and finer fibres. Furthermore, the seasons have no effect on the wool produced, provided the feed is kept constant. (Bosman, 1935.)

It has also been observed that when sheep, which have been grazed, are stall-fed, a reduction in the fibre thickness takes place. This latter effect may, therefore, be expected at Onderstepoort, where the sheep are stall-fed after having been reared under grazing conditions where they were bred.

The degree to which the various fibre characteristics respond, both individually and in relation to one another, calls for a systematic investigation, as it is felt that the result of such an investigation will be important, not only for its own sake, but also for its possible bearing on experimental results, obtained where similar conditions prevail.



In the present investigation no attempt has been made to assign or discuss possible causes of such variations in the wool produced. The object has been a statistical study of the data of the wool produced by a group of merino wethers sent from Grootfontein to Onderstepoort, where they were kept under experimental conditions for two years.

A point which should be borne in mind is that, in accordance with usual experimental procedure, the group of sheep was selected from the flock for uniformity of type, fleece and body weight.

In the study attention was directed mainly towards the following points:—

- (1) The change of each characteristic from one year to another.
- (2) The correlation between the values of the same characteristic for different years.
- (3) The correlation between different characteristics for the same year.
- (4) The correlation between the changes in different characteristics for the same sheep.

Such a study indicates to what extent the various fleece attributes are related to their previous values, and also the extent to which one attribute can be described in terms of another.

## II. MATERIAL.

### (a) *The Sheep.*

The sheep were obtained from Grootfontein and were used at Onderstepoort in a study of the effect of supplements of sulphur on growth and wool production. Since a full report of the experiment has been given (du Toit, Malan, Groenewald and Botha, 1935; van Wyk, Botha and Bekker, 1935), a brief summary of the history of the animals will suffice.

A selected group of merino wethers, born at Grootfontein between 10/3/1930 and 9/6/1930, were shorn between 31/3/1931 and 8/4/1931, and sent to Onderstepoort, where they arrived on 8/10/1931. They were shorn again in December, 1931, and placed on a basal ration for a pre-experimental period of eleven months. At the conclusion of this period in November, 1932, they were shorn again, and placed on the same basal ration for twelve months, during which period certain groups were dosed with supplements of sulphur and sulphur compounds. Finally, they were shorn at the end of the experimental period in November, 1933.

At Grootfontein the animals were grazed on typical Karroo veld, while the basal ration at Onderstepoort consisted of half a pound of crushed yellow maize and half a pound of green feed daily, with teff hay *ad lib*. Except in the case of the teff hay, individual feeding was employed.

The data were thus obtained for wool grown under the following conditions:—

1931: Six months grazing at Grootfontein, followed by two months at Onderstepoort, where the animals were stall-fed.

1932: Eleven months on basal ration at Onderstepoort.

1933: Twelve months on basal ration with sulphur supplements at Onderstepoort.

### (b) *Experimental Results.*

The sheep were weighed monthly from 23/12/31 until the conclusion of the experiment, i.e. during the pre-experimental and experimental periods. The grease weight ( $x$ ), clean weight ( $y$ ), and percentage yield ( $z$ ) of the fleeces obtained from the 1931, 1932 and 1933 clips at Onderstepoort were determined, as also the mean fibre length ( $l$ ), mean fibre thickness ( $t$ ) and mean fibre weight ( $w$ ) of a shoulder sample taken from an area marked on the skin. These samples were taken just before the sheep were shorn on each occasion.

From a statistical analysis of the results of the experiment (du Toit *et alia*, 1935; van Wyk *et alia*, 1935) it was concluded that, in the case of the wethers under consideration, the sulphur supplements had no influence on any of the fleece characteristics studied, hence for the purpose of this paper no differentiation between the groups will be made, and the data for the final period (1933) will be treated as if all the sheep received the same ration.

In the discussion the data for the separate years, 1931, 1932 and 1933 will be indicated by the suffixes 1, 2 and 3 respectively to the symbols for the attributes.

For the sake of direct comparison between the different years, the fleece weights are given as the average increase per day, while for fibre length and fibre weight the values given have been calculated so as to correspond to 300 days' growth. The fibre weights and fibre lengths for the 1931 samples were not available, and are, therefore, not included. The data for only 46 sheep were available for all three periods, hence this number was used in the analysis, except where otherwise stated.

In Table I are given the mean values of the fleece and fibre characteristics studied, together with the body weights and ages of the animals.

The figures for body weight and greasy fleece weight show that the sheep are a type which is mostly used at Onderstepoort in sheep experiments, the mature body weight being approximately 74 lb. and the fleece weight  $9\frac{1}{2}$  lb. for 12 months' growth.

TABLE I.

Period.	Age.	Mean Body Weight (shorn). (g)	Mean Fleece Weight (per day). (z)	Mean Scoured Fleece Weight (per day). (y)	Mean Fibre Length (Shoulder), (300 days). (l)	Mean Fibre Thickness (Shoulder). (t)	Mean Fibre Weight (Shoulder), (300 days). (w)
1931.....	Tooth. 2		11.567 ± 0.1567 gm.	6.065 ± 0.1802 gm.		16.76 ± 0.1638 μ	
1932.....	4	31.56 ± 0.2913 kg.	10.496 ± 0.1850 gm.	4.824 ± 0.0918 gm.	8.142 ± 0.0793 cm.	15.48 ± 0.1512 μ	19.99 ± 0.4289 10 <sup>-6</sup> gm.
1933.....	6	35.67 ± 0.3401 kg.	13.924 ± 0.2435 gm.	6.564 ± 0.1177 gm.	9.531 ± 0.1082 cm.	17.49 ± 0.1626 μ	29.79 ± 0.5753 10 <sup>-6</sup> gm.

TABLE III.

Characteristic.	$x_2 - x_1$	$y_2 - y_1$	$l_2 - l_1$	$t_2 - t_1$	$w_2 - w_1$	$g_2 - g_1$
Mean Difference.....	3.428 ± 0.1390 gm.	1.740 ± 0.0803 gm.	1.389 ± 0.0658 cm.	2.015 ± 0.0908 μ.	9.806 ± 0.4149 gm.	4.239 ± 0.4341 kg.
Percentage Change *.....	28.3	30.6	15.7	12.2	39.6	12.6

\* The change expressed as a percentage of the average for the two years.

The scoured fleece weight, representing the total amount of wool keratin produced by a sheep, is of primary importance, and depends on the total number of fibres and on the weights of the fibres. The weight of a fibre again depends on its thickness and length since the specific gravity is constant or very nearly so (King, 1926). The thickness and length of fibres can only be estimated from samples, and the values obtained vary with the area of the skin from which the sample was obtained. In merino sheep experiments it is usual to analyse a shoulder sample since this portion of the fleece represents the bulk of the fleece (Duerden and Bell, 1928).

Table I includes the fibre lengths and fibre weights calculated for 300 days' growth, together with the mean fibre thickness, in microns, of the shoulder samples. The average fibre thickness represents that of the dry fibre and has an average value of  $16.6\mu$ . The mean value for scoured fleece weight represents approximately five pounds of clean wool grown per year.

Table II gives the coefficients of variability (i.e. the standard deviation expressed as a percentage of the mean) of the various characteristics of the 46 sheep studied.

TABLE II.  
*Coefficients of Variability.*

Period.	Grease Weight (x).	Clean Weight (y).	Percentage Yield (z).	Fibre Length (l).	Fibre Thickness (t).	Fibre Weight (w).	Body Weight (g).
1931..	% 9.2	% 12.1	%	%	% 6.6	%	%
1932..	11.9	12.9	8.8	6.6	6.6	14.6	6.3
1933..	11.9	12.2	8.4	7.3	6.3	13.1	6.5

The table shows that there is a very close agreement between the coefficients of variability of the same characteristic for different years. Since the mean values vary considerably from year to year this would seem to indicate that the variation in each characteristic, as measured by the standard deviation, is proportional to the mean, so that the higher values are also more variable, and vice versa.

### III. COMPARISON OF THE DIFFERENT YEARS.

In Table I a considerable change is shown in the values of the fleece characteristics from one year to another. There was a decrease in all the values from 1931 to 1932 and then an increase in 1933 to values exceeding those of 1931. These changes took place throughout the group and are frequently observed in merino sheep that are similarly treated. Taking differences between the 1932 and 1933 values in order to eliminate correlational effects, the results summarised in Table III are obtained.

The percentage increases, therefore, varied from about 12 per cent. to 40 per cent. for the different characteristics, all these values being highly significantly different from zero. In the present paper the test for significance has been taken to indicate that the probability of obtaining a certain value or a greater one, on the assumption that the population value is zero, is less than 5 per cent, whereas "highly significant" is used to denote that the above probability is less than one per cent.

In the above case, therefore, all the differences may be considered as real and not merely accidental. These values, however, give no indication of how and when the changes occurred. It was, therefore, decided to obtain thickness measurements of a single staple taken from a sample at equal intervals of length. Although the results obtained in this way are only approximate, they show the general trend of the changes during the whole period of 3 years. A typical curve giving the values obtained for one sheep is given (Fig. 1). Several others were investigated and these showed remarkably close agreement with the one represented here.

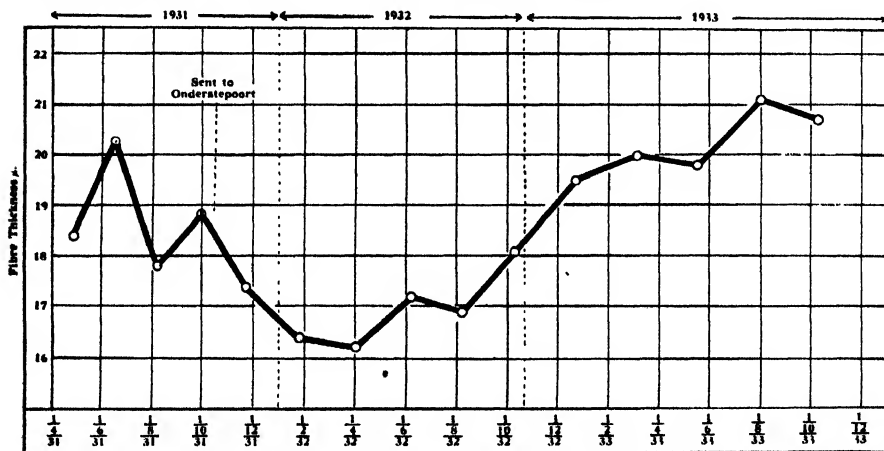


Fig. I.

It will be noted that the fibre thickness decreased to a minimum in April, 1932, followed by a continuous rise until July, 1933, when it seemed to have reached a more or less constant value, though no data are available after December, 1933. In other words, during the pre-experimental, or 1932 period, the fibre thickness at first diminished and then increased, while during the greater part of the experimental, or 1933 period, the thickness was increasing.

The bearing of the above changes on experimental results and their interpretation is of importance. Treatment effects which would normally have been reflected as small differences in fleece characteristics, may have remained obscure during the period when the fleece was undergoing wide changes, due to environmental factors. On the other hand, when a difference in treatment also affects the rate of adaptation to the new environment, the interpretation will greatly depend on the duration of the experimental period, and may lead to erroneous conclusions.

In the present case it took nearly two years for the fleece characteristics to attain values which appear to have reached a constant level under the changed environmental conditions. The rate and nature of the above changes need further investigation. For this purpose it seems advisable to send sheep from and to different centres under strictly controlled conditions, simultaneously eliminating other influencing factors, such as age, etc.

#### IV. ANALYSIS OF THE FLEECE.

(a) *The relation between the values for different years of the same attribute.*

The simple correlation coefficients,  $r_{12}$ ,  $r_{23}$  and  $r_{31}$  between the values of the fleece characteristics for the years 1931, 1932 and 1933 are summarised below:—

TABLE IV.

Correlation Coefficient.	Grease Weight (x).	Clean Weight (y).	Fibre Length (l).	Fibre Thickness (t).	Fibre Weight (w).	Body Weight (g).
1932 & 1933 : $r_{23}$ ..	<b>0.8235</b>	<b>0.7340</b>	<b>0.7682</b>	<b>0.8347</b>	<b>0.7436</b>	<b>0.4824</b>
1931 & 1932 : $r_{12}$ ..	<b>0.5108</b>	<b>0.7174</b>		<b>0.7811</b>		
1931 & 1933 : $r_{13}$ ..	<b>0.5286</b>	<b>0.5931</b>		<b>0.7891</b>		

(In some cases more animals than 46 were available and were used in calculating the above coefficients.)

*N.B.*—Significant values are given in italics and those highly significant are given in black type. This system is followed throughout in subsequent tables.

All the above correlation coefficients are highly significant, and practically of the same order, except perhaps in the case of body weights (*g*), where the correlation is somewhat lower. None of the values differ significantly, either amongst themselves, or between different years. The order of the correlation coefficients indicates that 50 to 60 per cent. ( $r^2 \times 100$ ) of the variation of a characteristic can be expressed in terms of the variation of the same characteristic for either of the two previous years. The high correlation coefficients, even when the change from one year to another is so exceptionally high, therefore demonstrate the necessity of a pre-experimental period in merino experiments where the fleece characteristics are being considered.

(b) *Simple correlation coefficients between different characteristics for the same year.*

It is evident from the agreement in the differences between the values from one year to another, as shown in §III, that the fleece weights at least are to some extent associated with fibre characteristics. The degree of interdependence, as expressed by the simple correlation coefficient, is shown in the table given below. The correlation coefficients between different characteristics have been calculated separately for each year.

TABLE V.

Characteristic.	Year.	Grease Fleece Weight (x).	Scoured Fleece Weight (y).	Fibre Length (l).	Fibre Thickness (t).
Scoured Fleece Weight (y)....	1931	0.6707			
	1932	0.8015			
	1933	0.7864			
Yield Percent (z).....	1932	-0.2623	0.4495		
	1933		0.4198		
Fibre Length (l).....	1932		0.3525		
	1933	0.3408	0.4471		
Fibre Thickness (t).....	1931	0.0088	-0.0591		
	1932	0.1647	0.2694	-0.0823	
	1933	-0.0601	-0.0729	-0.2214	
Fibre Weight (w).....	1932		0.4321	0.3889	0.8833
	1933		0.1821	0.3333	0.8431

The correlation coefficients between any two characteristics for the different years are in fair agreement, and indicate that the association between the different attributes, as measured by the simple correlation coefficients, is a constant feature in the wool of these wethers. This conclusion is on the whole strengthened by the values of simple correlation coefficients, calculated from the wool of 33 ewes, used in an iodine nutrition experiment (du Toit, Malan and Groenewald, 1935). These ewes were selected at Grootfontein from the same flock as the wethers in the present study, and were obtained at the same time. In the case of the ewes the following values for the correlation coefficients were obtained:—

TABLE VI.

	Clean Weight ( <i>y</i> ).	Fibre Length ( <i>l</i> ).	Fibre Thickness ( <i>t</i> ).
<i>l</i> , Fibre Length.....	0.1302		
<i>t</i> , Fibre Thickness.....	0.4631	0.0150	
<i>w</i> , Fibre Weight.....	0.4678		0.7065

The only exception as regards the agreement with the values in the previous table, Table VI, is that the correlation between scoured fleece weight (*y*) and the fibre characteristics, length (*l*) and thickness (*t*), is just the other way round. However, the real values of  $r_{yl}$  and  $r_{yt}$  are perhaps the same in both cases and intermediate between those obtained for the two groups of sheep. The discrepancy between the correlation coefficients given could also have been caused by the method of selection. Corresponding correlation coefficients calculated from 26 wethers (Bosman\*) agree well with those given in Table VI.

## V. VARIATION IN THE ATTRIBUTES FOR 1932 AND 1933.

It was shown in paragraph III that a considerable and highly significant change in the mean value of all the fleece characteristics studied took place from one year to the other. The order and nature of these changes have been discussed. It is also important to know to what extent the differences in individual values between consecutive years of associated attributes are correlated. The simple correlation coefficients are given below:—

TABLE VII.

	$y_3 - y_2$	$l_3 - l_2$	$t_3 - t_2$	$w_3 - w_2$	$g_3 - g_2$
$x_3 - x_2$ .....	0.8034				0.3590
$y_3 - y_2$ .....		0.6435	0.3337	0.3013	0.3306
$l_3 - l_2$ .....			0.2378	0.3405	0.0064
$t_3 - t_2$ .....				0.6511	-0.1784

\* Report in the course of preparation.



As may be expected a high correlation exists between the differences in grease weight ( $x$ ) and clean weight ( $y$ ). Two other high values are those between the differences in scoured fleece weight ( $y$ ) and fibre length ( $l$ ) and also fibre thickness ( $t$ ) and fibre weight ( $w$ ). Nearly all the other correlation coefficients are significant and practically the same with an approximate value of 0.33. The association between body weight differences and those of the fibre attributes is negligible. It follows, therefore, that in general a sheep that changes considerably in one attribute also changes likewise in another attribute.

The variability in fibre length and fibre thickness for each shoulder sample measured was calculated for 51 different sheep. The simple correlation coefficient between corresponding coefficients of variability for the same sheep was found to be 0.6226, which is a highly significant value. The simple correlation coefficient between the coefficients of variability of fibre length (shoulder sample) for the two years 1932 and 1933 was also calculated, and the value obtained for 45 sheep was 0.6402. This value is of the same order as the previous one and shows that sheep which are more variable in one characteristic are also more variable in another and remain so from one year to the other.

## VI. CONCLUSIONS.

From the preceding analysis, one is able to draw certain conclusions regarding the association of merino fleece and fibre characteristics.

It is evident that environmental conditions can produce considerable changes in the merino fleece. In the case under discussion, the fleeces at first became lighter and the fibres of a shoulder sample finer and shorter, after which the changes were reversed, and the initial values of the fleece and fibre characteristics exceeded. An increase from the second to the third period amounting to about 30 per cent. for the fleece and fibre weights was observed, and about half this value, approximately 15 per cent., for fibre length and fibre thickness, the difference between the mean values for the two periods, being highly significant.

Fibre thickness diminished rapidly after the sheep arrived at Onderstepoort, a minimum value being attained after about six months. A gradual increase then occurred to a probably constant value after a further period of fifteen months.

When, therefore, sheep are transferred from one centre to another, the change in environment may influence the fleece considerably, and a distinction should be made between this influence and that of any treatment to which the sheep are then subjected, and it must be recognised that the results obtained will depend on the nature of the possible interaction of the two influences. Otherwise it will be necessary to keep the sheep under the changed conditions, until the fleeces have attained what may be considered as normal characteristics, before commencing the experiment which is designed to ascertain the influence of some treatment or other on the wool.

As shown in Table V there exists a high correlation between the total fleece weight and the scoured fleece weight, the latter representing about 50 per cent. of the total fleece weight, whereas about 60 per cent. of the variation in the total fleece weight is due to the variation in the scoured fleece weight.

As regards the fibres of the shoulder sample, it is seen that the variation in the weight of a fibre depends mainly on the variation in its thickness, the variation in length having a relatively small influence on the variation in fibre weight. The correlation coefficients are constant from year to year, notwithstanding considerable changes in the actual values of the characteristics.

It is also demonstrated that the fibre attributes of a shoulder sample is a poor indication or no indication of the total amount of wool produced by a merino sheep.

The high correlation between the values of any of the fleece and fibre characteristics obtained for the different years is of importance. Approximately 60 per cent. of the variation in the values of one year can be expressed in terms of that for the previous year. This fact illustrates the importance of a pre-experimental period, which enables this proportion to be eliminated from the total variance, whereby a considerably greater accuracy is attained and smaller differences between mean values may be detected. In other words, a difference between treatment groups which would be obscured by the total variance, may become evident after an adjustment for the pre-experimental values has been made. For the same accuracy the number of sheep in any group of an experiment, without a pre-experimental period, must be approximately five times greater than when pre-experimental values are available.

The differences between the 1932 and 1933 values for all the fleece attributes were correlated. A difference in scoured fleece weight was closely reflected by a corresponding difference in the fibre length of the shoulder sample, whereas a difference in fibre weight was accompanied by a corresponding difference in fibre thickness. It is, therefore, possible to study a change in scoured fleece weight by means of the corresponding change in the fibre length of a shoulder sample.

Taking the group as a whole, the distribution of values, as expressed by the coefficient of variability, remained constant from year to year. In other words, the "spread" changed in proportion to the mean value.

A high correlation exists between the coefficients of variability of the fibre length of the shoulder samples taken from the same sheep in successive years. The variability, or spread, in fibre length is, therefore, a constant characteristic of individual sheep. For any one year, the correlation between the coefficients of variability of fibre thickness and fibre length is of the same high order, showing that a sample which is more variable as regards fibre length is also more variable as regards fibre thickness.

## VII. SUMMARY.

1. A statistical analysis is given of the fleece and certain fibre attributes of a shoulder sample of a group of wethers sent from Grootfontein to Onderstepoort for experimental purposes. The data comprise values obtained for three successive years.

2. It is shown that a considerable decrease occurred in the mean values from the first to the second year, and then again an increase to mean values exceeding those existing in the first year. The nature of the change in fibre thickness is given in more detail.

3. The values obtained for each characteristic in the different years are highly correlated, from which fact is deduced the importance of a pre-experimental period.

4. The correlation coefficients between the fleece weight and the fibre attributes are, though significant, not very high, and the shoulder sample is, therefore, a poor indication of the total amount of wool produced.

5. The ratios of the standard deviations to the corresponding mean values of the group were constant for the three years.

6. Within a shoulder sample a high correlation exists between the coefficients of variability of fibre length for two years, and also between the coefficients of variability of fibre length and fibre thickness for the same year.

7. The interrelationships of the changes in the various attributes are given.

8. The bearing of the above results on experiments, in which wool is being investigated, is discussed.

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## **Studies in Native Animal Husbandry.**

### **(13) A Note on the Three Parent Stocks of African Cattle.**

By H. H. CURSON, DR. MED. VET., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

IN a previous paper by Curson and Epstein (1934) illustrations were shown of the three parent stocks, that of the Hamitic Longhorn (which is apparently extinct) being taken from Kronacher's *Allgemeine Tierzucht*, 1 Abt., fig. 73. Kronacher gives Adametz as the source of this picture, which represents two Apis bulls from an ancient Egyptian text. As photos were not shown of the Brachyceros and Longhorned Zebu cattle as drawn by the Egyptians, illustrations will now be given and the opportunity taken to show the Hamitic Longhorn type, cows being represented as well as the bull.

#### **HAMITIC LONGHORN STOCK.**

Fig. 1 shows seven cows accompanied by a bull, both the conformation and colour patterns being very similar to that encountered to-day among Sanga cattle (which contain Hamitic Longhorn and Longhorned Zebu blood) in many parts of Africa. The picture is reproduced from Yahuda's *The Accuracy of the Bible*, and in referring to Pharaoh's dream, the author states: "As to the famous dream of the seven fat and seven lean kine (Gen. 41: 1-7), it was several years ago pointed out by Edouard Naville and others that such a story was only conceivable in Egypt, where the Goddess Hathor was worshipped in the form of a cow. As there were seven districts each having its Hathor cow, hence the seven kine. In the tomb of Nefretiry, the beautiful wife of Rameses II, the seven cows are to be seen accompanied by the bull-god as if they were marching in a solemn procession" (p. 8).

#### **BRACHYCEROS STOCK.**

Another representation of ancient cattle is shown in Fig. 2, where the Brachyceros type is depicted. Yahuda (1934) gives the source as "on the wonderful mural relief of the temple of Hatschep-sut in Dair-al-Bahri" (p. 8) and the legend under his picture is "Seven cows grazing in the 'Garden of Amon' in the meadow under frankincense-trees". Actually there appear to be nine head of cattle, the one in the foreground being a bull!

## LONGHORNED ZEBU STOCK.

Stegman von Pritzwald (1924) gives a figure (fig. 15, p. 56) which undoubtedly shows the Longhorned Zebu in Egypt during the New Kingdom (1580-945 B.C.\*). Epstein (1933) reproduces the picture in question, and as his figure lends itself better for photography, it is shown here as Fig. 3. Stegman von Pritzwald gives the source of his picture as W. M. Muller, but the original is not indicated.

Neffgen (1904), in his description of the Veterinary Papyrus of Kahun, refers to three ancient types of cattle in Egypt, viz.:—"Longhornrasse" (clearly Hamitic Longhorn), "Kurzhornrasse" (Brachyceros), and "Hornlosen Tiere". The same classification has been adopted by Carlier (1912) in his description of cattle in *L'Elevage au Kivu*,† but a classification based on the skull characteristics as a whole, rather than on the horns alone seems preferable.

Whereas there are also certain marked differences in the general body conformation of the Egyptian cattle (at any rate between the Longhorn and Shorthorn), the Kivu cattle (see Carlier, figs. 520, p. 778, and 527, p. 786) are apparently uniform except for the horn. This is what can be expected after the intermingling of centuries. A notable difference is that while the Egyptian cattle are humpless, those of the Belgian Congo have cervico-thoracic humps of apparently muscular tissue. The dewlap is also strongly developed in the cattle of Kivu. The influence of the Longhorned Zebu in this connection is significant.

An interesting feature of the Egyptian paintings is that cattle of the same type are selected, not crosses which must have been available.

Thanks are due to Miss D. Armstrong for drawing my attention to Yahuda's publication and to Mr. T. Meyer for the excellent photographs.

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\* Yahuda, p. xvii.

† And is also used by Edm. Leprieux in describing the native cattle of the Belgian Congo (*Organisation et Exploitation des Elevages du Congo Belge*, 2nd Edt., 1933).

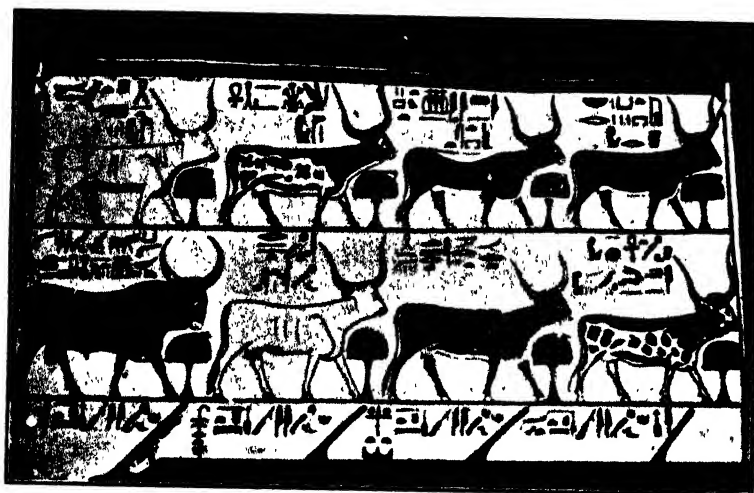


Fig. 1.

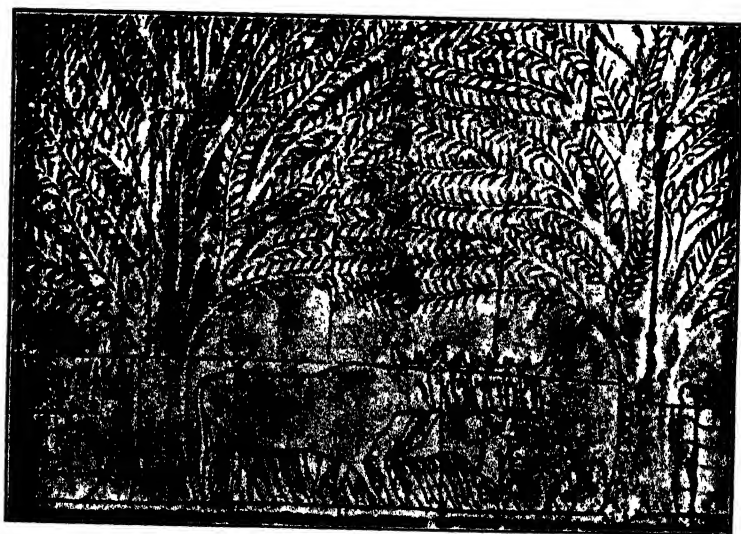


Fig. 2.



Fig 3.

## **Factors Affecting the Growth of Range Cattle in Semi-Arid Regions.\***

By

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### **INTRODUCTION.**

THE investigation reported in this paper deals with the comparative growth under range conditions of first generation offspring obtained by crossing cows of nondescript breeding with purebred bulls of five different beef breeds. The experiment was conducted at the Messina Ranching Station located in a semi-arid region of the Union of South Africa. Extensive areas in this region, which lies between 22° and 26° south latitude, are devoted almost exclusively to cattle raising.

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\* A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfilment of the requirements for the degree of Doctor of Philosophy.



The cattle common to this part of the country originally consisted of several fairly distinct types of indigenous cattle, but in recent years blood of the European breeds as well as that of the Afrikaner has been introduced into most of the herds, and interbreeding between different native types has occurred freely with the result that the cattle population at present consists of a heterogeneous mixture, showing some evidence of the presence of a small, but varying percentage of improved blood.

These cattle are relatively small and slow maturing as compared with the improved beef breeds. They lack beef conformation, being relatively long-legged, flat-ribbed and drooping in the rump. However the typical native cattle are extremely hardy and capable of subsisting on scant pastures. Moreover, they possess a high degree of tolerance to tick infestation and a resistance to tick-borne diseases.

No definite information is available on the origin of the indigenous types of African cattle, but it is generally accepted that they originated from a mixture of the Longhorn cattle (*Bos primigenius*) and the Zebu types (*Bos indicus*). The distinguishing characteristics of these species are still observed in various types of the present-day native cattle in the form of horn gigantism and hump respectively. Several distinct types of indigenous cattle have been described in detail by Groenewald and Curson (1933), Bisschop and Curson (1933), and others.

Attempts to improve the type and productiveness of these animals by means of grading up with purebred bulls of both dairy and beef breeds have not been conspicuously successful. Although the first generation obtained from such matings usually showed improvement in type and conformation over their dams, loss of constitutional vigour, failure to develop properly and increased mortality invariably have followed one or two further topcrosses with purebred bulls.

Since the reasons for the deterioration in size and constitution must be sought largely in the severe climatic conditions of this locality, the following brief description of the topography and vegetation is given of the experiment station where the investigation was carried out. The conditions at this station are fairly typical of the country. A granite formation dominates the entire area and the topsoil is shallow and exceedingly poor. The country is rough and hilly in most parts. The rainfall is low,—the mean annual precipitation for the years 1928-1932 was 14.20 inches—and there is practically no opportunity for growing supplementary forage crops. The greater part of the area lies at an elevation of 1,500 to 3,000 feet above sea level. The summers are extremely hot, shade temperatures of 100° F. to 110° F. being frequently recorded. Killing frosts rarely occur but the nights are chilly throughout the winter months.

The main characteristics of the pasture land are the scrub growth which forms a monotonous covering (see Appendix, figure 25) and the annual grasses. The dominant scrub types over most of the area are the Mapane (*Capaifera mopane*) and the species belonging to the family *Celastraceae* but in the more fertile sections several species of *Acacia* are prominent. The remainder of the vegetative covering

is composed of annual grasses intermixed with a small and varying percentage of perennial grass. On the poorest soil types and in overgrazed sections annual weeds are fairly prominent but on the hilly situations the perennial grasses are more abundant. It is therefore evident that a large proportion of the pasture has to establish itself annually.

*Enneapogon mollia* is the most commonly occurring grass. Other prominent annuals are *Aristida adsceniosis*, *Tragus racemosus*, and *Schmidtia bulbosa*. As regards perennial grasses the following are the most important economically: *Digitaria eriantha*, *Panicum maximum*, *Eragrostis atherstonii* and *Pennisetum cenchroides*.

The comparatively small perennial grass growth is largely due to the low annual rainfall and unfavourable soil conditions. Although the soil is composed largely of decomposed granite there is apparently no serious mineral deficiency as determined by chemical analysis of samples of soil and of the vegetation.

## OBJECTS OF THE INVESTIGATION.

At the time the investigation was started the main objects in view were to determine the degree to which native cattle could be successfully graded up under practical range conditions by the continued use, in successive generations, of purebred bulls. For this purpose it was proposed to make a comparative study of the five major beef breeds found in the country by breeding these to unimproved cows and on their progeny for several generations.

A secondary object was to determine the most suitable practice of herd and pasture management. However, this phase of the investigation suffered a severe setback after the work had been in progress for two years owing to an outbreak of foot-and-mouth disease in the adjoining territories with consequent necessary quarantine restrictions.

The present study is concerned with the growth of the first generation animals obtained from this scheme of breeding.

## REVIEW OF LITERATURE.

The various aspects of the subject of growth are comprehensively reviewed in recent publications by Robertson (1923), Robbins and co-authors (1928), Brody and Associates (1926), (1927), and Hammond (1932). Growth in dairy cattle has been extensively studied but comparatively few systematic investigations on the growth of range beef cattle have been reported.

Moulton and associates (1921) investigated the growth of beef steers on different planes of nutrition from birth to 1,440 days of age. Their results indicate that animals fed to the limit of their appetites show a remarkably uniform increase in weight up to the age of 1,200 days. Animals fed on a plane of nutrition to furnish maximum growth without fattening show a similar curve except that the rate of gain is slower. Animals fed scantily to the extent where growth

was actually retarded show a more irregular curve with a perceptibly slower average rate of growth. However, it was found that the scantily fed animals reached the same wither height as the full fed ones at the age of four years. The authors concluded that a greater underweight than underheight resulted from the feeding of poor rations. They also note that growth is more rapid at the earlier ages.

Hogan and Fox (1923) present growth curves for beef steers fed in such a manner as to secure as rapid growth as practicable under ordinary conditions. These curves show a uniformly straight line up to the age of two years when the downward trend, indicating a decreasing growth rate, commences.

Clawson (1926) in a study of the weight of range cattle on pasture found that the younger animals made larger percentage gains than the older ones and that the rate of gain was most rapid in the early part of the season and gradually slowed down in the autumn. These observations were made during a period of four years from June to September.

In his studies on type in beef calves Hultz (1927) found very marked changes in body proportions or "type" during a six months feeding period. He also states that the most important measurements for the determination of type are depth of chest, paunch girth and height at withers.

In a comprehensive review of the growth studies on dairy cattle at the Missouri Agricultural Experiment Station, Brody (1927) states "weight and girth are apparently affected to nearly the same *relative* extent by conditions of undernutrition . . . one reason is that the measurement of circumference of chest includes not only the skeletal growth but also the flesh around the chest . . . The height at withers measurement does not measure fleshiness". He concludes that the measurements which approach their mature values at relatively rapid rates are but slightly influenced in their growth by environmental conditions as compared with the influence of the same conditions on those which approach their mature values at a relatively slow rate as compared with weight.

Lush (1928) has shown that certain measurements, noticeably those of width, are greatly affected by degree of finish or fatness apart from growth. Eckles and Swett (1919) found no relation between size at birth and rate of growth or size at maturity. They also state that gestation has no effect on growth whereas lactation and nutrition have pronounced effects on body weight. These factors also influence the rate of growth in wither height to some extent but have no effect on the height attained at maturity. They observed that the rate of growth in skeleton is the same for such widely different breeds as Holsteins and Jerseys up to 24 months of age. Espe, Cannon and Hansen (1932) also observed a pronounced effect of lactation on growth in weight in dairy cattle.

Brody and Ragsdale (1921) found evidence of two post-natal cycles in the growth of Holstein and Jersey heifers, the maximum growth velocity in the first cycle occurring at four and a half to five months and the second, an ill-defined maximum at 20 months.

Hammond (1920) collected data from the records of the Smithfield Club of stock exhibited at Fat Stock shows and studied the relative growth of various breeds and crosses of cattle. He analyzed the weights for animals at different ages but these cannot be considered as normal growth since exhibition stock are usually fed and managed exceptionally well. However, he observed definite breed and sex differences for the breeds studied.

In his studies on the growth of the lowland cattle of Eastern Prussia, Hansen (1925) found that the younger the animals were the greater was the rate of growth in all directions. The measurements of width increased most rapidly, followed by measurements of length and finally measurements of height. He found that growth of various measurements ceased between the ages of three and four years. He also observes that the plane of nutrition of the animals during the early stages of development is very important.

Ashton (1930) in presenting growth measurements of various European breeds of cattle makes the following interesting observation: "It would seem that in general the various European breeds of cattle vary in volume or size according to the quality or nature of the soil where they are born and reared. . . . The Brittany, truly a dwarfed breed in every respect, owes its extremely small size to the natural lack of both calcium and phosphorus in the geological formation of those parts of Brittany where the breed was evolved."

The importance of minerals, especially phosphorus, in the normal growth of range cattle on phosphorus-deficient pastures, was investigated by du Toit and Bisschop (1929) in an extensive series of experiments. Cows of the nondescript unimproved type were bred to four different breeds of bulls. The herds were kept under natural range conditions except that certain groups were fed small amounts of bonemeal daily. Regular weights were taken but only two sets of body measurements were taken at two different ages on a number of half-bred heifers of different breeding in each group. The following conclusions were drawn in regard to the growth of these animals:

"The influence of bonemeal on the *rate of growth* of calves is remarkable. At birth there is no difference between 'bonemeal' calves and 'control' calves; at six months there is a difference of 48 lb. (or 14.5 per cent.) in favour of the former group; and at two and a half years the difference is 243 lb. (or 32.2 per cent.)."

In regard to the effect of bonemeal feeding on skeleton growth the authors state: "There is a marked difference in favour of cows receiving bonemeal in respect to length of body, width of chest, heart girth and width between hook bones. The skeletal development of bonemeal-fed calves is also superior in every way to the control calves."

It was also observed that the increase in weight of the young stock showed a definite seasonal trend depending upon the condition of the pastures, consequent upon the rainfall. Growth usually received a check during late winter to early summer, i.e. from July to December.

In regard to breed differences it was found that the Sussex half-breeds were heavier at six months than all other breeds (Afrikander, Red Poll, Friesland) and that they retained this lead throughout the period of the experiment. The Red Poll half-breeds weighed considerably less than the other groups at six months but soon overtook the Afrikander and Friesland. The latter groups showed approximately the same rate of increase.

The most extensive investigation on the growth of range cattle under conditions of yearlong grazing is that conducted by Lush and co-workers (1930). This investigation covered a period of nine years during which an extensive series of body measurements in addition to live weights were taken. The following breeds and crosses were included in the study: Herefords, first cross high grade Brahman-Hereford, and backcrosses of the latter to the Hereford. The conclusions drawn from this study may be summarized as follows:—

The most rapid rates of increase in weight occurred from mid-April to mid-July, after which they gradually decreased until mid-January to early March when actual losses were recorded. The growth curves show a definitely seasonal trend and variations from the typical rates of growth are directly connected with rainfall and pasture conditions.

Measurements greatly influenced by degree of fatness were affected to a greater extent by seasonal changes than measurements of the head and of the long bones .

Breed and sex differences were relatively unimportant but quarter-blood Brahmans were slightly heavier than half-bred Brahmans or high grade Herefords. Steers were slightly heavier than heifers at all ages up to 30 months.

In regard to the effect of adverse conditions on ultimate size the authors conclude as follows: "The evidence indicates rather clearly that the skeletal growth is really slowed down in parts of the body by the winter period of scanty feed. Whether mature size is permanently stunted at all by winter periods of feed shortage or is only postponed to a later age than would be the case with cattle well fed the year round is not clear. These data and the data from the experiments at other stations lead us to believe that very little if any of such permanent stunting occurs."

## PLAN OF EXPERIMENT.

In 1928 two hundred cows of the unimproved type were purchased from farmers in the surrounding country and these were transferred to the experiment station near Messina, Northern Transvaal. Selection of the cows was confined to such factors as freedom from disease or visible defects; a fairly good frame and reasonable thriftiness but no special attention was paid to the breeding of the animals. Some showed evidence of an infusion of Afrikander blood while others showed the presence of either Friesland, Shorthorn or Hereford blood in earlier generations. The average type of these cows is illustrated in Appendix figures 26 and 27.

Two bulls of each of the following breeds were acquired: Hereford, Shorthorn, Sussex, Aberdeen-Angus, and Afrikaner, as representing the five major beef breeds used extensively in South Africa. The Afrikaner is a breed developed from indigenous strains of cattle and has been fully described by Bosman (1924), Reinecke (1927) and du Toit and Bisschop (1929). The information on the origin of the breed is fragmentary. However, all available information tends to show that it was developed from the large framed cattle of the Hottentots, a tribe of African native which inhabited the coastal regions of the Cape at the time of the advent of the white man in the early eighteenth century. The Hottentot cattle were considered a distinct type differing widely from that of the cattle owned by the Bantu tribes. Epstein (1933) has recently endeavoured to show the similarity in many respects of the present day Afrikaner to the early Hottentot cattle and he concludes that there can be little doubt that this breed traces back ultimately to the *Bos indicus* of Asia. It is reasonably certain that the European breeds of cattle played very little or no part in the formative period of the Afrikaner since it had already become established as a breed at the time of the first importation of European cattle towards the end of the eighteenth century. On the other hand, evidence of a Zebu (*Bos indicus*) origin is displayed in the characteristic hump and sloping rump. Although the Afrikaner Cattle Breeders' Society was only established in 1912 and the registration of animals only dates from that time, the breed had been bred pure for many generations at the time of the outbreak of the Anglo-Boer War in 1899 and many notable herds were in existence at that time. As the result of the war most of these herds became scattered and valuable breeding records became lost so that the pedigrees of the present-day Afrikanders generally cannot be traced further back than the beginning of the present century. As a breed the Afrikaner may be described as medium of size and inclined to be narrow and shallow of body. It is somewhat leggy, judged from the standpoint of a beef animal, but it shows very fine, clean bone. It is relatively slow in development and both sexual maturity and maximum body growth are attained at a later age than is commonly found in the British breeds of beef cattle. The colour is red, ranging from light to deep cherry red and the coat is short and sleek. The breed is noted for its hardiness, rustling ability and resistance to ticks and tick-borne diseases.

The selection of the bulls was carried out in co-operation with the respective breed Societies in order to ensure that the animals would be representative of the average type of ranching bull. They were medium priced bulls and cannot be considered representative of the best of their respective breeds. The type of these bulls may be observed in the illustrations in Appendix figures 28 to 32. They were immunized against Redwater (Piroplasmosis) and gall sickness (Anaplasmosis) before being introduced into the area.

The cows were branded and divided at random into five groups of equal numbers and each group was bred to bulls of one of the breeds mentioned above. It was planned to breed each group of cows to all five breeds of bulls in rotation in successive years.

*Management.*—In view of the dense bush and the absence of adequate fencing it was extremely difficult to keep the herds apart during the breeding season and to prevent animals from straying. Consequently all herds were rather closely herded by day and kraaled\* at night.

During the breeding season the groups were kept separate and the bulls were allowed to run with their respective herds every day from late afternoon until the following morning, for a period of approximately 10 weeks. The breeding season was restricted with the object in mind of limiting the calving season to the months of April, May and June. At the conclusion of the service period the groups were combined into two large herds which were allowed to graze on the various sections of the station in rotation.

All calves were ear-tagged at birth and branded some time after weaning. The males were castrated (bloodlessly) by means of the Burdizzo Pincers before they were three months old. Weaning occurred at an average age of eight months. After weaning the calves were run in one herd until they were 18 months old when the steers and heifers were separated.

No supplementary feeding was practised at any time during the year except in the case of the bulls. These received small supplements of cottonseed meal, crushed maize and at odd intervals a small allowance of hay during the service period. Salt and bonemeal were fed at irregular intervals. Generally the animals showed no craving for salt, due to the high sodium chloride content of the drinking water which was obtained from boreholes.

In view of the fact that this part of the country was heavily infested with ticks and due to the prevalence of Anaplasmosis, Piroplasmosis and Heartwater all cattle were dipped regularly at weekly intervals during the summer months and bi-weekly during the winter months.

## EXPERIMENTAL DATA AND METHODS.

Commencing in January 1931, all calves were weighed regularly at bi-monthly intervals from birth. The calves born in 1929 and 1930 were weighed at birth and again at weaning time. Thereafter they were also weighed at bi-monthly intervals. The foundation cows were weighed only once a year when their calves were weaned. At the first weighing in January 1930, they were weighed on three consecutive days and the average of the three weights recorded. For all other animals only single weights were recorded.

In January 1931, a system of taking body measurements was adopted. All half-bred steers were measured at bi-monthly intervals up to May, 1933. Measuring of the females was discontinued approximately three months prior to freshening. The method employed in taking measurements was as follows: A group of animals was placed in a pen and the individual to be measured was driven into a crush pen where it was secured with cross beans. A concrete platform

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\* Confined in a small enclosure or coral.

had been constructed on the floor of the pen to ensure that the animal would stand at the same level with all four feet. An attempt was made to stand the animal in a natural position but this was extremely difficult in some instances, especially in the case of the young stock as some animals were extremely wild. Linear measurements were taken with a "Deriaz" measuring stick graduated in inches, and circumference measurements were taken with a tape measure similarly graduated.

These operations were usually carried out at the middle of the month but as numbers grew the process occupied several days. The following body measurements were taken:—

Height at withers—the vertical distance from the ground to the highest point over the withers.

Height over hips—the vertical distance from the ground to the highest point midway between the hooks.

Depth of chest—the smallest vertical distance behind the shoulders.

Depth of flank—the vertical distance between the highest point in the rear flank and the top of the body just in front of the hook bones.

Width of chest—the greatest width of the chest immediately behind the shoulders.

Width at thurls—the horizontal distance between the hip points.

Width at hooks—the horizontal distance between the lateral points of the hooks.

Width of loin—the horizontal distance between two corresponding points midway between the anterior point of the pelvis and the last rib.

Length of pelvis—the distance between the anterior point of the hooks and the pin bones on the posterior.

Length of body—the distance from the shoulder point to the pin bone taken parallel with the axis of the body.

Heart girth—the smallest circumference of the body immediately behind the shoulders.

Paunch girth—the greatest circumference of the body perpendicular to the navel or sheath.

Flank girth—the smallest circumference of the body at the rear flank, immediately in front of the hooks.

Width between pin bones.

The last mentioned measurement proved to be very inaccurate owing to the difficulty in locating the pin bones exactly, especially in the well-fleshed or fat animals. Consequently these figures have been discarded and this body measurement is not being used in this study. For the same reason other measurements taken from the pin bones to some other point, e.g. length of pelvis and length of body are subject to greater error than the rest of the measurements. In addition the measurement of length of body is also affected by the



standing position of the animal. The same also applies to height at withers. All other linear measurements are not subject to these sources of error to any marked extent and are therefore reasonably accurate. Measurements of circumference are affected by the tautness with which the tape is drawn around the body and since it is difficult to judge this accurately these measurements are also subject to an appreciable error in this respect. In addition, these measurements, especially paunch girth, are greatly affected by the "fill" of the animals.

All body measurements of the individual animals were taken at the same time and recorded in a form provided with columns under the respective headings. Subsequently the observations for each body measurement were arranged according to year of birth, breed and sex.

The relevant data for three groups of animals, born in 1929, 1930 and 1931 respectively, are used in the present study. It should be noted that all the animals are half-breds of the different breeds of bulls used but occasionally they will be referred to simply as "Herefords", "Shorthorns", "Aberdeen-Angus", etc., in the following pages for the sake of brevity.

### ANALYSIS OF RESULTS.

In the following section the results are analysed from the standpoints of general growth and breed and sex differences. In the analysis of the latter the method of analysis of variance introduced by Fisher (1933) has been used. This method is particularly well adapted to a study of this nature in view of the relatively small number of observations involved and the disproportionate frequencies in the sets. The computations were made on a Monroe Calculating machine after the method of Wallace and Snedecor (1931). The test of significance is effected by a direct comparison of the mean squares through the use of Snedecor's (1934) table for values of F for the corresponding degrees of freedom. These values bear a close relation to those of Z in Fisher's Table VI (1933). This relationship may be expressed as follows:—

$$\begin{aligned}\frac{\text{Variance I}}{\text{Variance II}} & \dots\dots\dots = F \text{ (Snedecor's notation).} \\ \log_e \text{ Variance I} - \log_e \text{ Variance II} & \dots\dots = \log_e F. \\ \log_e \text{ Variance I} - \log_e \text{ Variance II} & \dots\dots = 2 Z \text{ (Fisher's notation).} \\ \log_e F & \dots\dots\dots = 2 Z. \\ F & \dots\dots\dots = e^2 Z.\end{aligned}$$

The standard errors of the mean differences have been derived directly from the standard deviation of the observations from their respective breed means as follows:—

$$\text{Standard Error} = \text{S.D.} \sqrt{\frac{1}{n_1 - 1} + \frac{1}{n_2 - 1}}$$

in which S.D. is the standard deviation *within* groups and  $n_1, n_2$  are the numbers of animals respectively in the groups compared.

## LIVE WEIGHTS.

The average weights of all half-breds born in 1929, 1930 and 1931 respectively are shown in Appendix Tables XXIII to XXV. The weights are compared according to year of birth, breed and sex. For purposes of comparison the average weights of the dams of the 1929 group are also included.

The average birth weights of all animals classified according to sex are shown in the following table.

TABLE I.

*Average Birth Weights in Pounds of all Half-breds.*

Sex.	No.	Sex Ratio.	Breed.					
			Hereford.	Short-horn.	Sussex.	Afri-kander.	Ab.-Angus.	Average.
Males.....	176	% 52.87	68.2	67.8	70.9	63.2	57.7	65.0
Females.....	157	47.13	63.2	60.7	66.3	57.9	56.7	60.8
Average...	—	—	65.8	63.3	68.7	60.0	57.2	63.1

These figures do not differ materially from those reported by du Toit and Bisschop (1929) for range cattle of similar breeding but they are appreciably lower than those reported by Lush and co-workers (1930) for Hereford and Hereford-Brahman crossbred range cattle.

The difference between the sexes is highly significant as shown in Table II. The value of  $F=15.06$  may be compared with that corresponding with Fisher's .01 probability which is 6.72 for the corresponding degrees of freedom. Likewise the differences between breeds are highly significant for both sexes. The corresponding values of  $F$  designated as highly significant are 3.43 and 3.45 for males and females respectively. In the lower half of the table the breeds are arranged in descending order in respect of birth weight and mean differences with their respective standard errors given between the different breeds. It will be observed that the ranking of the breeds is the same for both sexes. In the case of the males both Sussex and Herefords proved significantly heavier than the Afrikanders and the Aberdeen-Angus, while the Shorthorns and the Afrikanders are significantly heavier than the Aberdeen-Angus. In the case of the females the Sussex are significantly heavier than all breeds except the Herefords. The latter are significantly heavier than both Afrikanders and Aberdeen-Angus.

TABLE II.  
*Analysis of Variance of Birth Weights.*

Classification.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.*
Sex.....	Total.....	332	33,356.32	—	—	—
	Between sexes..	1	1,452.00	1,452.00	—	—
	Within sexes...	331	31,904.32	96.39	9.8	<b>15.06</b>
Breed : (a) Males...	Total.....	175	25,140.77	—	—	—
	Between breeds	4	9,885.02	2,471.25	—	—
	Within breeds..	171	15,255.75	89.21	9.4	<b>27.71</b>
(b) Females.	Total.....	156	12,445.69	—	—	—
	Between breeds	4	1,676.70	419.18	—	—
	Within breeds..	152	10,768.99	70.85	8.4	<b>5.91</b>

## MEAN DIFFERENCES.

		Hereford.	Shorthorn.	Afrikander.	Ab.-Angus.
Males.....	Sussex.....	2.69±2.3	3.14±2.5	<b>7.76±2.2</b>	<b>13.20±2.1</b>
	Hereford.....	—	0.45±2.5	<i>5.07±2.2</i>	<b>10.51±2.2</b>
	Shorthorn.....	—	—	4.62±2.4	<b>10.06±2.4</b>
	Afrikander.....	—	—	—	<b>3.44±2.1</b>
Females.....	Sussex.....	3.12±2.3	5.63±2.5	8.33±2.2	<b>9.64±2.1</b>
	Hereford.....	—	2.51±2.5	<i>5.21±2.2</i>	<b>6.52±2.2</b>
	Shorthorn.....	—	—	2.70±2.4	4.01±2.4
	Afrikander.....	—	—	—	1.31±2.1

\* NOTE.—Significant differences ( $P = .05$ ) are given in italics and those highly significant ( $P = .01$ ) are given in black type. This system is followed throughout in subsequent tables.

*Growth in Body Weight.*—Live weights are used most commonly to depict growth in animals. There are several methods of constructing growth curves from such data. The usual method is to plot the actual weights against age or time. This gives the absolute growth curve as shown in Appendix figures 9 to 11 in which the weights of the different breeds for each sex have been plotted against age. These charts show the general trend of the weights of each breed of the different groups for both sexes separately and indicate the seasonal fluctuations in the weights. However they do not convey a correct picture of the relative rate of growth. Several other methods may be used. Minot (1908) originally suggested the "Simple Interest" method which may be represented by the formula

$R = \frac{W_2 - W_1}{W_1}$  where  $R$  is the relative rate of growth and  $W_1$  and  $W_2$  represent successive weights. Brody (1927) suggested two different equations for relative growth rate at different ages: First, for growth

preceding the inflection at puberty, represented by the formula  $K = \frac{dW/dt}{W}$ , and second, for the phase of growth following the inflection the following formula is proposed  $K = \frac{dW/dt}{(A-W)}$  where  $W$  is weight at the given instant,  $(A-W)$  growth yet to be made, and  $k$  proportionality constant or relative rate of growth.

However, the formula proposed by Fisher (1921) appears to be the best adapted to the study of relative growth rates and this method has accordingly been used in the present study. In Fisher's formula  $R = \frac{\log_e m_2 - \log_e m_1}{t_2 - t_1}$  where  $m_1$  and  $m_2$  are successive weights and  $t_2 - t_1$  represents the length of the interval between weighings.  $R$  is the relative growth rate.

Figure 1 shows the relative (instantaneous) growth rates expressed in percentage from weaning of steers and heifers of all breeds in the 1929 group.

It will be observed that there is little change in the relative rate of growth between the sexes until the age of 34 months. From this age up to the age of 42 months the steers show an appreciably greater relative increase (or smaller loss) in weight than the heifers. The former age coincides with the time that the heifers first came into lactation. Upon weaning of their calves the former were 42 months of age and the relative rate of increase again rises to that of the steers. At the age of 46 months there is a further decrease in the growth rate of the heifers. At this age they came into lactation again. This situation is also clearly brought out in Figure 2 in which the average bi-monthly weights are plotted in semi-log paper. These curves show the relative growth of the sexes throughout the period and bring out the divergence in the lines after the age of 32 months is reached.

It is of course well known that steers are normally heavier than heifers at maturity but this sharp divergence must be ascribed partly to the effect of lactation on the females. Eckles and Swett (1919) and also Espe, Cannon and Hansen (1932) found that while gestation had no effect on growth rate, lactation exercised a pronounced influence. When the females are divided into two groups on the basis of whether or not they had been in lactation the following results are obtained:—

TABLE III.  
*Effect of Lactation on Body Weight.*

Group.	Number.	Weight.	
		July, 1931.	January, 1933.
Lactating.....	26	709	790
Non-lactating.....	21	705	903
DIFFERENCE.....	—	4	—113

FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

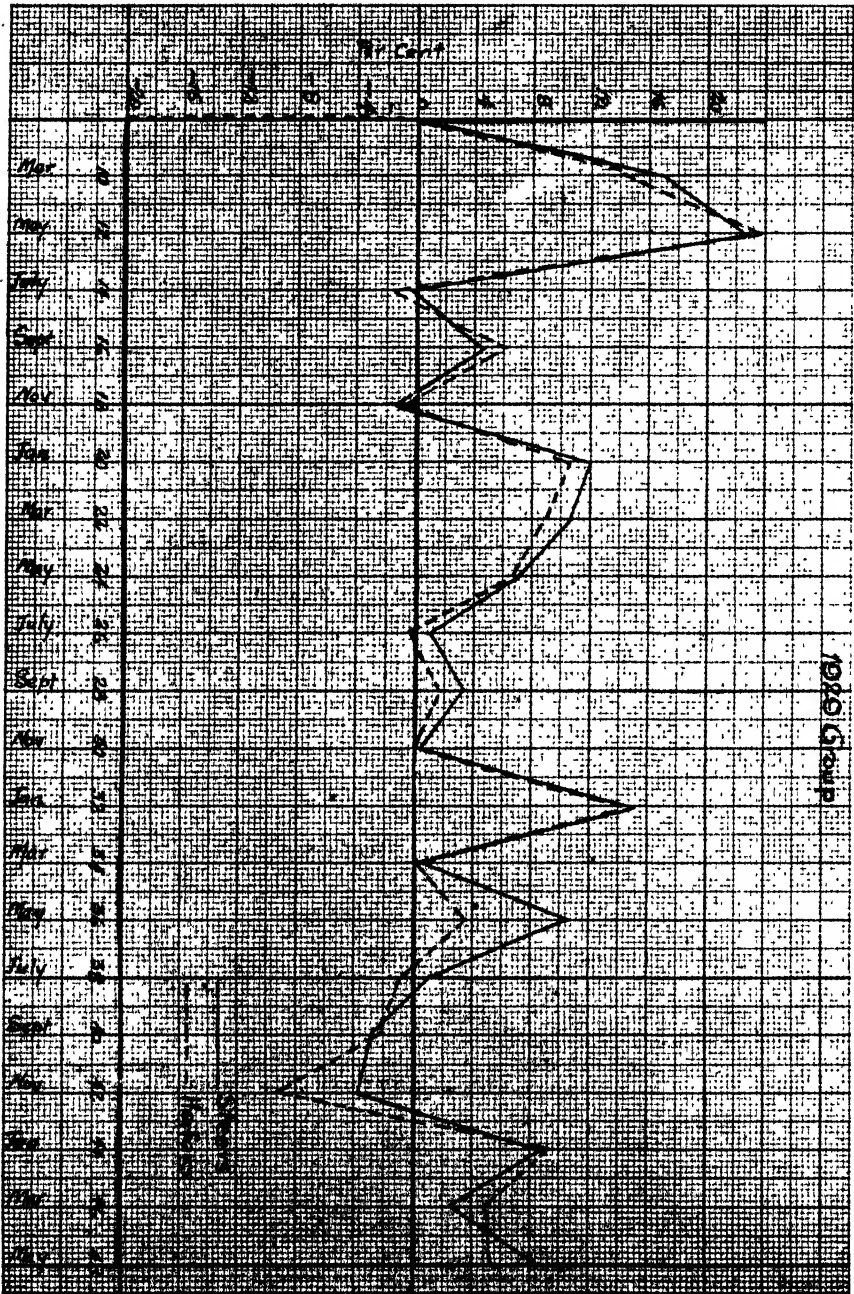


Fig. 1.—Percentage growth rates. Live weights of 1929 steers and heifers.

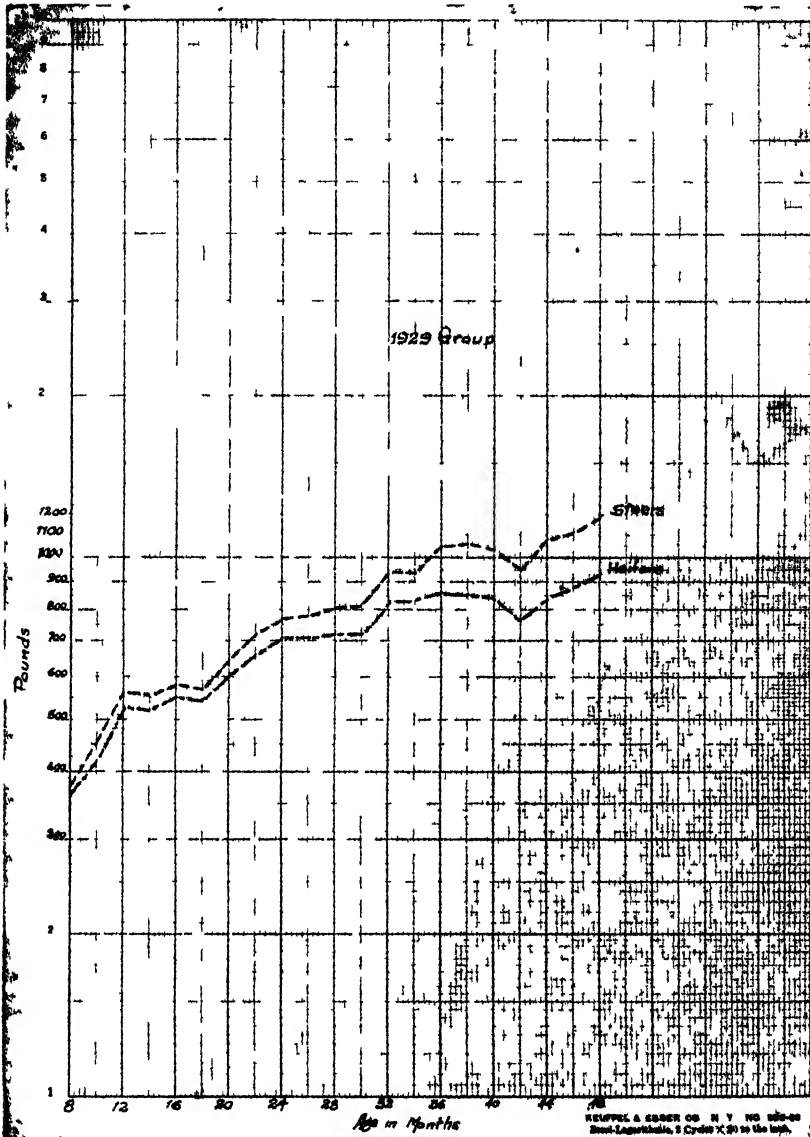


Fig 2 —Relative growth Live weights of 1929 steers and heifers

When the difference in the last column is tested for significance by the method of analysis of variance a value  $F=21.64$  is obtained, which is highly significant. The difference in the heights at withers of the above groups in January 1933, was similarly tested. Although the non-lactating group showed the higher average height the difference is only on the borderline of significance ( $F=2.61$ ). Lactation evidently retarded growth in height to some extent especially in view of the fact that the animals were still immature.

*Breed Differences.*

Appendix Tables XXIII to XXV show the average weights for the different breeds grouped according to year of birth. Appendix figures 9 and 10 show the average trend of the changes in weight, from weaning up to the ages of 51 months and 39 months respectively, for the 1929 and 1930 groups, while the average trend of the weights of the 1931 group from birth up to 27 months of age is indicated in Appendix figure 11. It will be observed that all breeds follow more or less the same course and it would appear that environmental factors, such as climatic conditions and feed supply, have the same general effect on all breeds. This point will be further discussed in a later section.

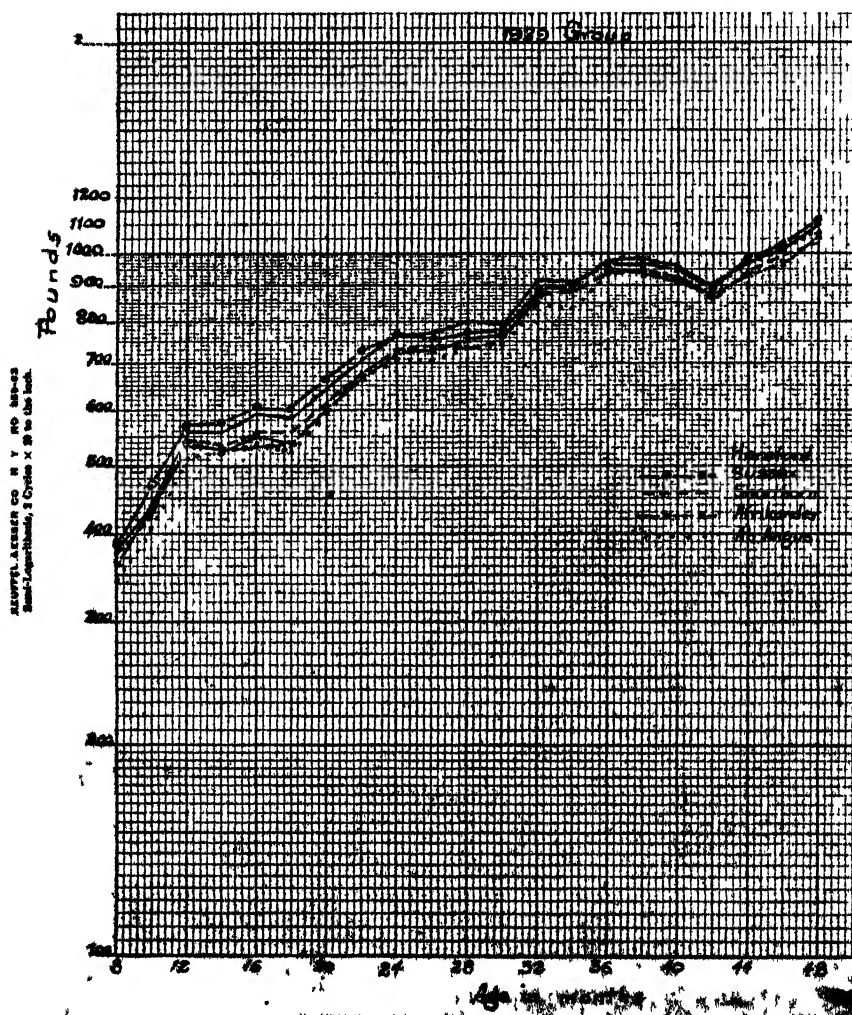


Fig 3.—Relative growth of the 1929 group. Live weight.





# FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

36 months, the breeds maintain their relative positions throughout the period and generally speaking there is practically no difference between any of these breeds in this respect. However, the differences in absolute weights between breeds in all three groups are not inconsiderable.

The differences are analysed separately for the sexes in Tables IV-VI.

TABLE IV.

*Analysis of Variance of Final Weights. 1929 Half-breds.*

	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
Steers.....	Total.....	49	499,966.6	—	—	—
	Between breeds	4	180,774.6	45,193.6	—	—
	Within breeds..	45	319,192.0	7,093.2	84.2	<b>6.37</b>
Heifers.....	Total.....	44	403,850.5	—	—	—
	Between breeds	4	93,416.0	23,354.0	—	—
	Within breeds..	40	310,434.5	7,760.86	88.1	<b>3.01</b>

## MEAN DIFFERENCES.

		Afrikander.	Shorthorn.	Sussex.	Ab.-Angus.
Steers.....	Hereford.....	<b>115±35.8</b>	<b>125±42.7</b>	<b>153±37.8</b>	<b>158±34.4</b>
	Afrikander.....	—	10±42.7	38±37.8	43±34.4
	Shorthorn.....	—	—	28±44.3	33±40.6
	Sussex.....	—	—	—	5±34.4
		Shorthorn.	Hereford.	Ab.-Angus.	Afrikander.
Heifers.....	Sussex.....	84±45.4	<b>132±44.0</b>	<b>132±49.0</b>	<b>137±45.4</b>
	Shorthorn.....	—	48±37.7	48.0±42.5	53±39.4
	Hereford.....	—	—	0.0	5±37.7
	Ab.-Angus.....	—	—	—	5±42.5

The value of  $F=6.37$  in the case of the steers is highly significant. The corresponding value for the one per cent. probability being  $F=3.77$  for 45 degrees of freedom. In the tests of significance of mean differences in the lower half of the table the breeds have been arranged in descending order of their mean weights.\*

The Herefords prove to be significantly heavier than all other breeds while no heterogeneity is demonstrated among the other breeds. The conclusion is reached that the high value obtained for  $F$  is due entirely to the extraordinary high average weights attained by the Hereford half-breds, namely 1,312 pounds at 48 months of age as compared with 1,197, 1,187, 1,159 and 1,154 for the Afrikander, Shorthorn, Sussex and Aberdeen-Angus half-breds respectively.

\* This system is followed in all subsequent tests of significance of mean differences between breeds.

In the case of the females heterogeneity between breeds is likewise demonstrated, the value  $F=3.01$  being above the 5 per cent. point. However in this case the Sussex are significantly heavier than all breeds while the differences between the rest of the breeds are not significant. A possible explanation for the high average weight of the Sussex as compared with the Herefords may be sought in the fact that only 50 per cent. of the former freshened during the previous year as against 83 per cent. in the case of the latter. Similarly, the low average weight of the Afrikaner half-breeds may be accounted by the high calving percentage of 83 per cent. as compared with 50 per cent. for each of the remaining breeds.

TABLE V.

*Analysis of Variance of Final Weights. 1930 Half-breeds.*

	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
Steers.....	Total.....	53	451,761	—	—	—
	Between breeds	4	65,232 9	16,308 2	—	—
	Within breeds..	49	386,528 1	7,888 3	87 7	2 06
Heifers.....	Total.....	52	286,968	—	—	—
	Between breeds	4	56,828	14,207	—	—
	Within breeds..	48	230,140	4,794 6	69 2	2 96

## MEAN DIFFERENCES.

		Sussex.	Afrikaner.	Ab.-Angus.	Shorthorn.
Steers.....	Not significant.				
Heifers.....	Hereford.....	1 0 ± 32 9	43 ± 26 4	69 ± 29 6	88 ± 31 6
	Sussex.....	—	42 ± 31 4	68 ± 33 4	87 ± 35 8
	Afrikaner.....	—	—	26 ± 27 9	45 ± 29 0
	Ab.-Angus.....	—	—	—	19 ± 32 8

In the 1930 steers the differences between the breeds are not significant. However, the Sussex and the Hereford half-breeds are again appreciably heavier than the other breeds. It will be observed that the value of  $F=2.06$  borders on significance, the value corresponding to the 5 per cent. point being  $F=2.56$ .

In the 1930 females the difference is significant. In this case the Herefords and the Sussex are significantly heavier than the Aberdeen-Angus and the Shorthorn while the differences between the latter are not significant.

The animals of the 1931 group were only 20 months of age on the average when the final weights were taken. The difference between breeds is highly significant for both sexes, the values for  $F$  being above the one per cent. probability. In the case of the steers the Herefords and the Sussex are again significantly heavier than the remaining three breeds. The mean differences between all other breeds are not significant.

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In the case of the females all breeds are significantly heavier than the Afrikanders while the differences between the rest of the breeds are not significant.

TABLE VI.

*Analysis of Variance of Final Weights. 1931 Half-breeds.*

	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
Steers .....	Total .....	63	278,400	—	—	—
	Between breeds	4	73,935.27	18,483.82	—	—
	Within breeds ..	59	204,464.73	3,465.50	58.8	<b>5.32</b>
Heifers .....	Total .....	52	187,566	—	—	—
	Between breeds	4	63,976	15,994	—	—
	Within breeds ..	48	123,590	2,574.8	50.7	<b>6.21</b>

## MEAN DIFFERENCES.

		Sussex.	Afrikander.	Shorthorn.	Ab.-Angus.
Steers .....	Hereford .....	10±22.8	62±22.5	<b>75±25.4</b>	<b>82±22.5</b>
	Sussex .....	—	52±21.3	65±24.2	<b>72±21.3</b>
	Afrikander .....	—	—	13±25.1	20±22.5
	Shorthorn .....	—	—	—	7±25.1
		Shorthorn.	Hereford.	Ab.-Angus.	Afrikander
Heifers .....	Sussex .....	9±26.2	10±21.0	35±23.3	<b>38±21.0</b>
	Shorthorn .....	—	1±24.7	26±26.7	<b>79±24.7</b>
	Hereford .....	—	—	25±21.7	<b>78±19.2</b>
	Ab.-Angus .....	—	—	—	53±21.7

It is concluded from the analysis of variance of the weights taken at different ages on the three groups of cattle that the breeds may be classified into two major weight groups namely, Hereford and Sussex in one group and Shorthorn, Afrikander and Aberdeen-Angus in the second group. While the differences in average weight between breeds within each of these groups are not significant, the breeds in the first group are significantly heavier than those in the second group.

## Seasonal Influence on Live Weight.

No evidence of the peculiar cyclic nature of growth in weight as described by Brody and Ragsdale (1921) is shown by the growth curves of any of the three groups. This is possibly due to the extreme effects produced by the seasons of the year which would tend to mask the expression of such cycles especially in the older animals. The absolute growth figures indicate the same general trend and seasonal fluctuations for all groups. Referring again to Figure 4 which shows the relative percentage growth rate of all animals in each of the five breeds for the 1929 group from weaning up to 4 years of age, it will be observed that the highest percentage increase occurred at the

age of 12 months, the next peak is between 20 and 22 months, the third at 32 months and the fourth at 44 months. These ages coincided with the following months of the year respectively: May 1930, January-March 1931, January 1932, January 1933. The ages at which relative growth was the least (actual losses being recorded in most instances) are as follows: 18 months, 26 months, 34 months, and 42 months. These ages coincided with the following months respectively: November 1930, July 1931, March 1932, November 1933.

The fluctuations in relative growth rate between periods of greatest and smallest gains respectively is very considerable and clearly demonstrates the influence of season on the weights of cattle at any age from weaning up to maturity. The fluctuations in the growth of the 1930 and the 1931 groups exactly coincide in a vertical plane with those described above. Thus in the 1930 group the peaks occur in January-March 1931 (age 8-10 months), January 1932 (age 20 months), January 1933 (age 32 months). Likewise in the 1931 group the peaks occur in January 1932 (age 8 months), and January 1933 (age 20 months).

It is therefore apparent that growth in live weight is strictly seasonal under the conditions obtaining in the locality where these cattle were raised. Most rapid growth occurs during the late summer and early autumn months while growth is retarded to a great extent during late winter and early spring. Rainfall rather than season *per se* is the cause of these fluctuations. The rainfall is limited and strictly seasonal. The heaviest precipitation usually occurs from November to February. The monthly rainfall for the five-year period during which this experiment was in progress is given in Table VII.

TABLE VII.  
*Monthly Rainfall in Inches.*

	1928.	1929.	1930.	1931.	1932.	1933.
January.....	1.64	3.07	3.72	0.16	2.56	10.88
February.....	2.08	5.88	0.30	0.60	4.21	—
March.....	0.43	0.89	1.60	1.43	5.77	0.90
April.....	—	—	1.80	1.35	1.47	—
May.....	—	0.10	0.40	—	0.06	—
June.....	—	0.05	—	—	—	—
July.....	—	—	—	1.10	—	—
August.....	0.16	0.02	—	—	—	—
September.....	—	—	0.08	—	—	—
October.....	0.11	0.65	—	1.66	0.71	—
November.....	1.77	4.68	2.69	2.94	1.30	—
December.....	2.43	1.94	4.22	1.45	3.11	—
TOTAL.....	8.62	17.68	14.81	10.69	19.19	11.78*

\* 6 months total.

The rainfall for the 4-month period November to February inclusive was 12.15; 9.64; 7.67; 11.16; 15.24 inches, as compared with 0.80; 1.71; 5.88; 5.54; 8.31 inches for the remaining 8-month

period for the years 1928 to 1933 respectively. These figures show more clearly the reason for the relatively large percentage increases each year. It will be observed that there was practically no precipitation during the period extending from May to October hence the retardation in growth of body weight up to November.

That this is the general situation in the semi-arid regions of South Africa in the summer rainfall area is fully borne out by the results obtained by du Toit and Bisschop (1929) in which they invariably observed an actual loss in weight in young growing cattle during the period July to November as compared with uniformly substantial increases during the period December to May.

Similar results have also been obtained by Lush and co-workers (1930) for range cattle in Texas. They record actual losses in weight from mid-January to mid-March (corresponding with the season July to November in the present study) and the greatest increase in weight from mid-April to mid-July (corresponding with the season December to March).

These results are in strong contrast to those obtained with cattle kept on a uniform level of nutrition throughout the year as reported by Moulton and associates (1921) and Hogan and Fox (1923) for beef steers and by Brody and Ragsdale (1923), (1925) for dairy cattle. Under these conditions the animals show a uniform, although slightly decreasing rate of growth throughout the year from birth up to the age of three years or over.

Returning to Appendix Table XXV, attention is drawn to the similarity between the final weights of the 1929 half-bred heifers and those of their dams. The average weights of the Shorthorn, Sussex and Afrikaner half-breds are slightly higher than the averages of their respective dams, while those of the Hereford and Aberdeen-Angus half-breds are slightly lower than those of their dams. These differences are small, however, and it must be concluded that the half-bred females show no significant improvement in body weight over their dams. The steers, on the other hand, are uniformly heavier than their dams but it is difficult to determine accurately whether or not this is due to normal sex differences as no data are available on the weights of steers of the unimproved type. The question arises as to whether the use of pure-bred beef bulls on the native cows has resulted in any marked improvement in weight of the offspring over that of their native dams. Any possible effect of lactation on the weights of the half-bred offspring must be ruled out since the weights of the dams represent the average of weights taken in January in three successive years just at the time when their calves were weaned. Several possible explanations suggest themselves but discussion of these must be deferred until the body measurements have been examined.

## BODY MEASUREMENTS.

### *Growth in Body Measurements.*

As pointed out in a previous section the measurements were discontinued in the case of the heifers shortly after they had been bred so that completed figures are available only for the steers.

Furthermore, these measurements were only initiated in the beginning of 1931 consequently no data are available for the 1929 group up to the age of 20 months, nor for the 1930 group prior to the age of 8 months. However, the animals of the 1931 group were measured at bi-monthly intervals from birth up to the age of 24 months so that there is sufficient overlapping of the measurements at different ages in the different age groups to enable one to estimate with a fair degree of accuracy the nature of the continuous relative growth in body shape.

The relative growth of different parts of the body is presented graphically in Figures 5 and 6. Figure 5 shows the average linear measurements of all 1931 steers from 2 months up to 24 months of age. Figure 6 shows the same measurements for all 1929 steers from the age of 24 months up to 48 months. All body measurements were plotted on the same logarithmic scale consequently these curves also show the relationship of the different body parts to each other.

All measurements show fairly rapid growth in the pre-weaning stage. At the age of 8 months there is a slight downward deflection of the curves. This deflection becomes more marked at the age of 12 months. From this point the curves straighten out indicating a continuous but decreasing rate of growth up to the age of 24 months. Figure 6 shows the relative growth in the older animals. The various body measurements occupy the same relative position in the diagram as in the case of the younger group. Practically the same relative growth is shown throughout the period up to the age of 48 months when the measurements ceased.

#### *Seasonal Influence on Body Measurements.*

Although some measurements, notably those of width (between hooks, between thurls; chest and loin width) show some irregularity, in no case are such excessive fluctuations observed as in the case of body weight (see Figure 3). The curves representing body length, hip height and wither height respectively, are particularly smooth. In Figures 7 and 8, which represent the percentage growth rates of the 1931 and 1929 groups respectively, the measurements have been divided into those which show the greatest and those showing the least seasonal fluctuations respectively. The former comprise the following measurements in descending order of the degree of fluctuation: width of chest, width of loin, width at thurls, width at hooks, and length of pelvis. The second group includes depth of flank, width of chest, length of body, height over hips and height at withers. The former group of measurements occupy the lower half of the respective diagrams and the latter occupies the upper half.

Figure 7 shows at a glance the course of the percentage growth rates in the young animals. The growth rate for all measurements in the upper half of the diagram diminishes rapidly until the age of 14 to 16 months. It remains fairly constant during the succeeding four months except in the case of flank depth which shows rather wide fluctuations, and thereafter again shows an upward trend. The same general trend is observed for the measurements in the lower half of the diagram. In the latter, however, the fluctuations are much greater and such measurements as width of chest and width of loin actually show substantial negative growth rates at the age of 14 months.

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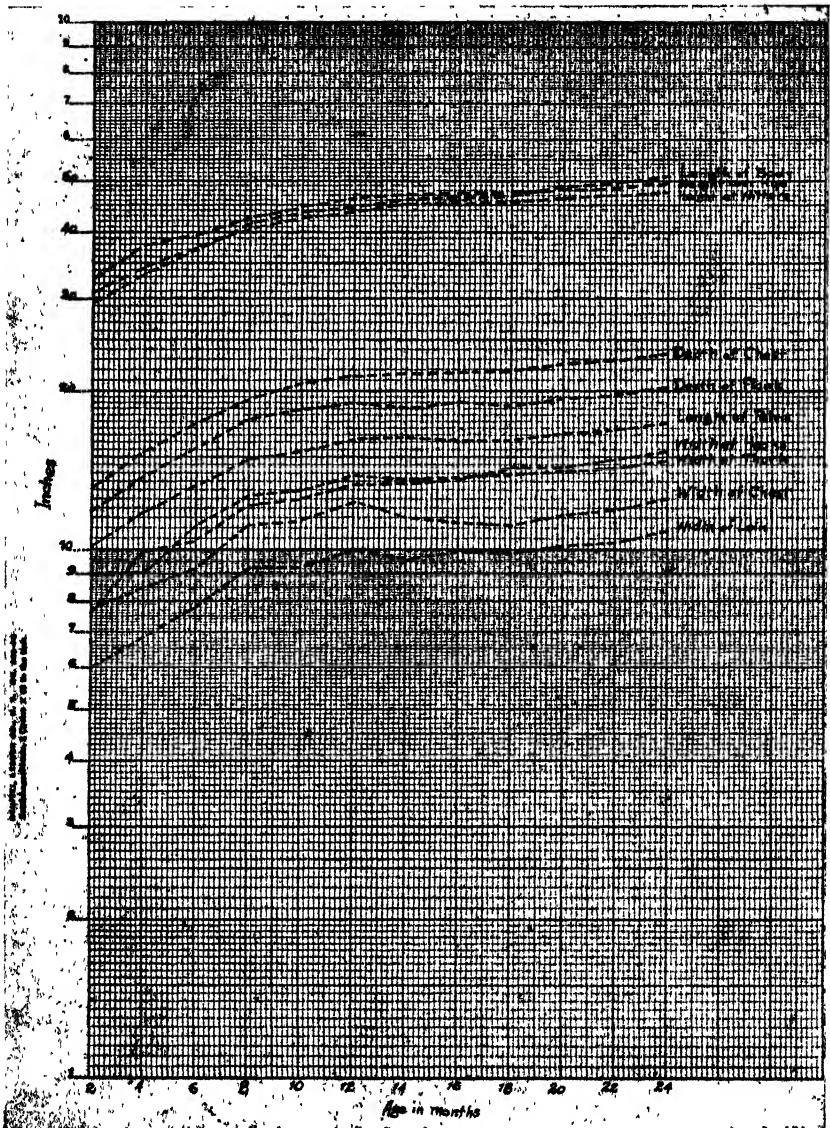


Fig. 5.—Relative growth in various body dimensions. 1931 steers.

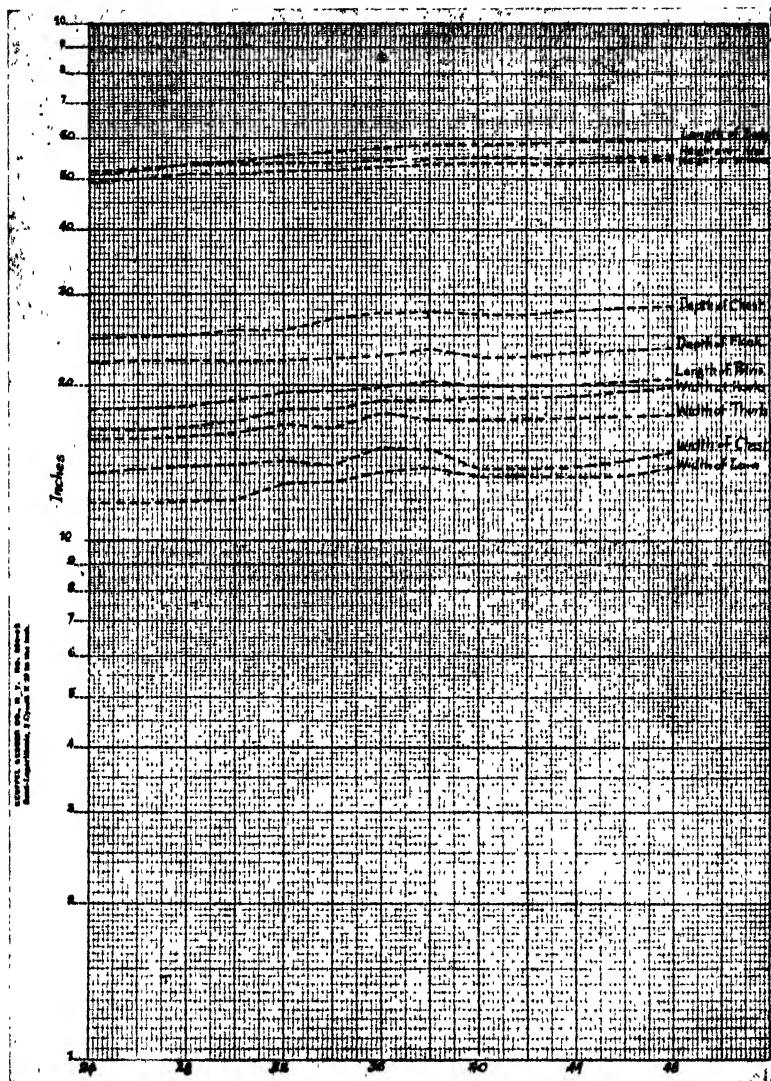


Fig. 6.—Relative growth in various body dimensions. 1929 steers.



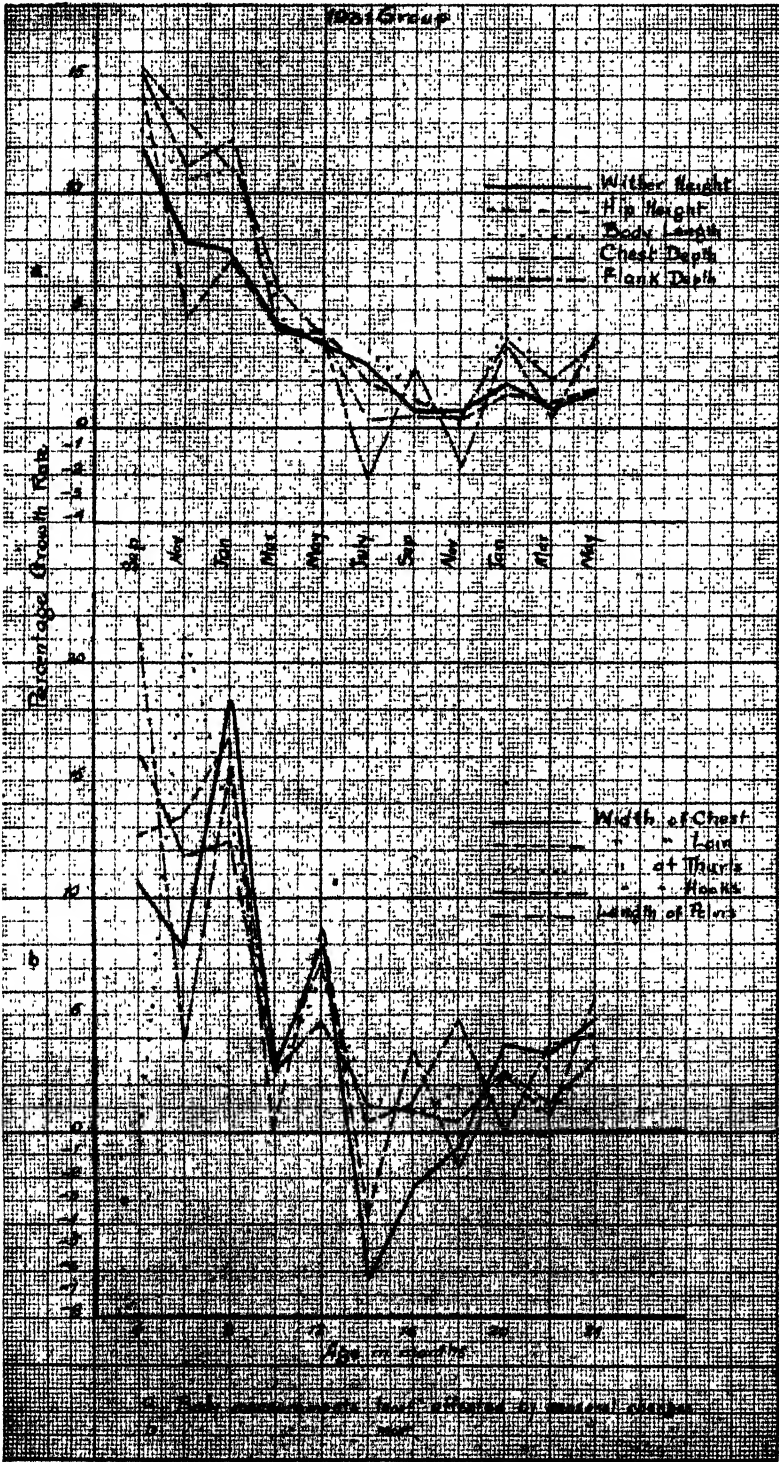


Figure 8 shows the corresponding measurements for the older group of steers. The measurements in the upper half of the diagram show a relatively constant, although slightly diminishing, rate of growth. Those in the lower half show wide fluctuations from month to month and tend to diminish at a greater rate with increasing age. Substantial negative values are again observed in the case of the latter.

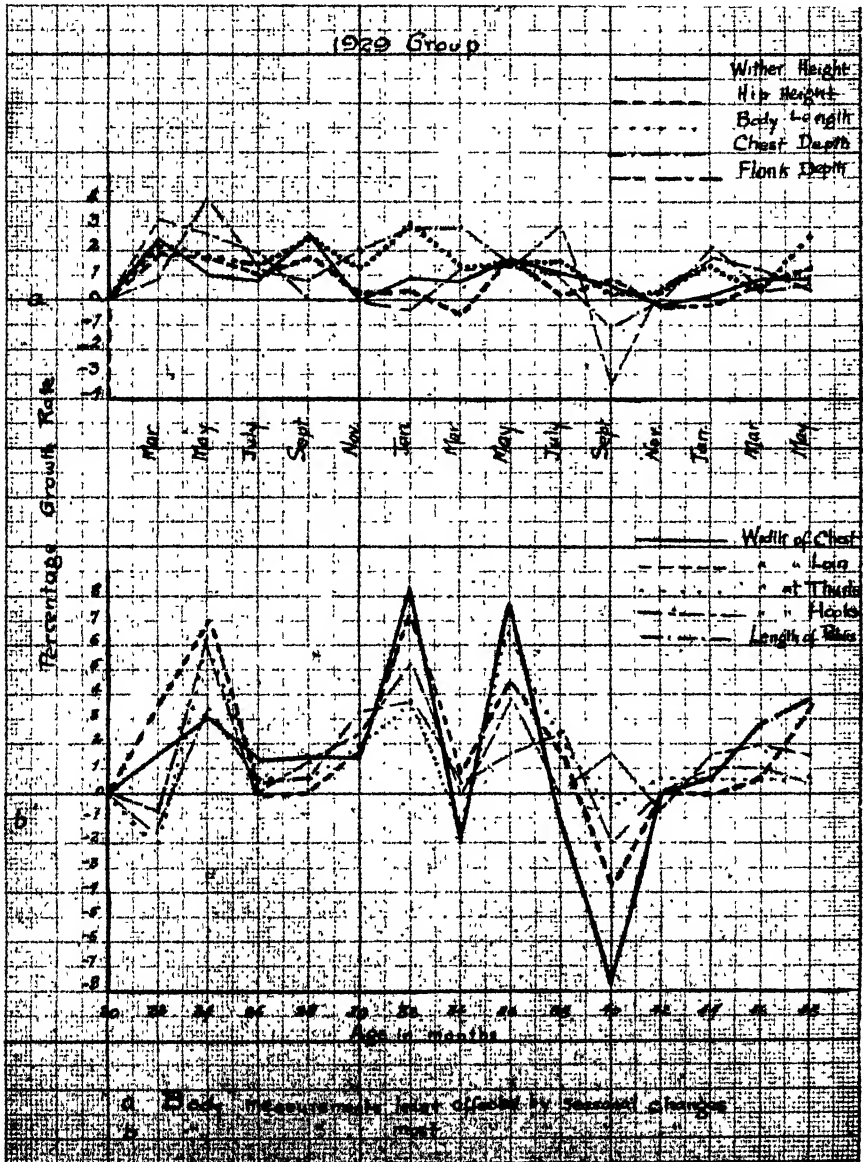


Fig. 8.—Percentage growth rates of various body dimensions. 1929 steers.

The ages at which the rate of growth in body measurements was highest correspond exactly with those observed for the greatest increase in weight as shown in Figure 4. Similarly, the points of smallest or negative growth rate coincide with those at which losses in weight occur. These fluctuations must therefore be attributed largely to seasonal influences. As pointed out by Lush (1928) certain body measurements are greatly affected by the degree of fleshing or fatness in the animals.

It can be reasonably assumed that the seasonal fluctuations in the rate of growth in respect to both weight and body measurements are due primarily to the alternating periods of abundance and scarcity in the feed supply. The production of forage in this region is largely dependent upon frequent precipitation in view of the temporary nature of the grasses which consist predominantly of annual species. Under conditions of a uniformly optimum feed supply such fluctuations in growth do not occur as pointed out by Moulton and co-workers (1921), Hogarty and Fox (1923) and Lush and co-workers (1930). Even if no permanent stunting in ultimate size of the animals occurs, interference with the normal development of the animals results and such interference undoubtedly influences the age of maturity. It is generally held that the native cattle in the semi-arid region of South Africa are late maturing. Our own observations confirm this view and it is suggested that this phenomenon is indirectly the result of inadequate nutrition and that the presence of natural selection in favour of a type of animal that can subsist on a low plane of nutrition is stronger than man's artificial selection for beef conformation.

### *Breed Differences.*

The measurements of the steers are presented graphically in Appendix Figures 12 to 24. The three groups of curves in each diagram have been so arranged that a perpendicular line intersecting the horizontal axes of all three at any point represents the same date (month and year) so as to give a comparative picture of seasonal influences on the various body measurements of different breeds at different ages. It will be observed that the course of the absolute measurements is very similar for all breeds indicating that the breeds are affected by conditions of environment such as feed supply, more or less to the same extent. In the younger age groups the relative differences in certain body measurements between the breeds appears at a comparatively late stage in the development of the animals. The differences between breeds in the different age groups of steers are analyzed in the following section.

Referring to Table VIII it is seen that no heterogeneity is demonstrated in either the 1931 or the 1930 group. In fact the variance within breeds is greater than that between breeds in either group. This may be due to age differences in the animals within the groups although it should be pointed out that the calving season was controlled within certain limits and the extreme range in ages between individual animals is generally less than two months. However the values of  $F=1.55$  and  $F=1.22$  are insignificant. On the other hand, in the 1929 group the difference between breeds is highly

significant;  $F=5.04$  as compared with a value of 3.77 for a probability of one per cent. against the difference being due to experimental error. The breeds rank in the following descending order: Afrikaner, Shorthorn, Hereford, Aberdeen-Angus and Sussex.

TABLE VIII.

*Analysis of Variance of Height at Withers. Final Measurements.  
Half-bred Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	143.5273	—	—	—
	Between breeds	4	7.9556	1.98	—	—
	Within breeds..	59	135.5717	2.29	1.51	1.55
1930.....	Total.....	53	99.8330	—	—	—
	Between breeds	4	6.2819	1.57	—	—
	Within breeds..	49	93.5511	1.91	1.38	1.22
1929.....	Total.....	49	167.2621	—	—	—
	Between breeds	4	51.7701	12.94	—	—
	Within breeds..	45	115.4920	2.57	1.59	<b>5.04</b>

MEAN DIFFERENCES.

1931.....	Not significant.				
1930.....	Not significant.				
1929.....	Afrikaner.....	Shorthorn. 0.54 ± 0.81	Hereford. 0.61 ± 0.68	Ab.-Angus. <b>2.36 ± 0.66</b>	Sussex. <b>2.83 ± 0.72</b>
	Shorthorn.....	—	0.07 ± 0.81	1.82 ± 0.77	2.29 ± 0.84
	Hereford.....	—	—	1.75 ± 0.66	2.22 ± 0.72
	Ab.-Angus.....	—	—	—	0.47 ± 0.69

The first three breeds are significantly higher over the withers than the Aberdeen-Angus and Sussex. The differences between all other breeds are not significant.

Table IX shows the analysis of variance of height over hips. The breed differences prove to be highly significant in each age group, the corresponding values for F for the one per cent. point being 3.65, 3.72 and 3.77 as compared with 4.39, 3.68 and 5.47 for the three groups respectively.

Inspection of the mean differences between individual breeds indicates that the Afrikanders are significantly higher over the hips than the Aberdeen-Angus in all age groups. In the 1931 group the Afrikanders are also significantly higher than the Shorthorns; in the 1930 group they are significantly higher than the Herefords, and in the 1929 groups significantly higher than the Sussex. In the 1931 group both Sussex and Herefords are significantly higher than the Aberdeen-Angus and in the 1929 group the Herefords and the Shorthorns are significantly higher than the Aberdeen-Angus. In each case the Afrikanders show the greatest height over hips and the Aberdeen-Angus the least height except in the 1930 group where the Herefords show a slightly lower figure than the Aberdeen-Angus.

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The other breeds occupy intermediate positions and show no consistency in their ranking with respect to each other from one age group to the next.

TABLE IX.

*Analysis of Variance of Height over Hips. Final Measurement.  
Half-bred Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	186 4131	—	—	—
	Between breeds	4	42 8085	10.70	—	<b>4.39</b>
1930.....	Within breeds..	59	143.6046	2.43	1.56	—
	Total.....	53	125.1861	—	—	—
	Between breeds	4	28.9496	7.24	—	<b>3.68</b>
1929.....	Within breeds..	49	96 2365	1.96	1.40	—
	Total.....	49	163.7089	—	—	—
	Between breeds	4	53.5725	12.39	—	<b>5.47</b>
	Within breeds..	45	110.1364	2.45	1.56	—

## MEAN DIFFERENCES.

		Sussex.	Hereford.	Shorthorn.	Ab.-Angus.
1931.....	Afrikander.....	0.43±0.56	0.68±0.60	1.40±0.67	<b>2.22±0.59</b>
	Sussex.....	—	0.25±0.60	0.97±0.64	<b>1.79±0.56</b>
	Hereford.....	—	—	0.72±0.67	<b>1.54±0.60</b>
	Shorthorn.....	—	—	—	0.82±0.67
1930.....		Shorthorn.	Sussex.	Ab.-Angus.	Hereford.
	Afrikander.....	0.59±0.60	0.80±0.58	1.16±0.54	1.25±0.62
	Shorthorn.....	—	0.21±0.64	0.57±0.61	0.66±0.68
	Sussex.....	—	—	0.36±0.57	0.45±0.66
1929.....	Ab.-Angus.....	—	—	—	0.09±0.63
		Hereford.	Shorthorn.	Sussex.	Ab.-Angus.
	Afrikander.....	0.82±0.67	1.00±0.79	<b>2.08±0.70</b>	<b>2.75±0.64</b>
	Hereford.....	—	0.18±0.79	1.26±0.70	<b>1.93±0.64</b>
	Shorthorn.....	—	—	1.08±0.82	1.75±0.76
	Sussex.....	—	—	—	0.67±0.68

It should be noted that the relative positions of the breeds in respect to height over hips are very similar to those for height at withers.

Inspection of Table X shows that the differences in body length between breeds is significant only in the 1929 group, although the values of F in the other groups approach significance. Referring to the mean differences between individual breeds in the lower part of the table it is quite apparent that the high value obtained for F in the 1929 group is due entirely to the exceptionally great length of

body of the Herefords as compared with the other breeds, the former being significantly longer than all other breeds. The differences between all other breeds are not statistically significant.

TABLE X.  
*Analysis of Variance of Body Length.*  
*Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Free-dom.	Sums of Squares.	Mean Squares.	Standard Devia-tion.	F.
1931.....	Total.....	63	234 0000	—	—	—
	Between breeds	4	26 2691	6 56	—	1.86
	Within breeds..	59	207 7309	3 52	1.88	—
1930.....	Total.....	53	225 2697	—	—	—
	Between breeds	4	30 9083	7 72	—	1.95
	Within breeds..	49	194 3614	3.96	1.99	—
1929.....	Total.....	49	283 9293	—	—	—
	Between breeds	4	60 0425	15 01	—	<b>3.03</b>
	Within breeds..	45	223 8868	4 97	2.23	—

MEAN DIFFERENCES.

1931.....	Not significant.				
1930.....	Not significant.				
1929.....	Hereford.....	Ab.-Angus.	Shorthorn.	Afrikander.	Sussex.
	Ab.-Angus.....	$2.49 \pm 0.91$	$2.76 \pm 1.13$	$3.18 \pm 0.95$	$3.21 \pm 1.00$
	Shorthorn.....	—	$0.27 \pm 0.08$	$0.69 \pm 0.91$	$0.72 \pm 0.97$
	Afrikander.....	—	—	$0.42 \pm 1.13$	$0.45 \pm 1.17$
		—	—	—	$0.03 \pm 1.00$

TABLE XI.  
*Analysis of Variance of Depth of Chest.*  
*Final Measurement of Steers.*

Group.	Source of Variation.	Degrees of Free-dom.	Sums of Squares.	Mean Squares.	Standard Devia-tion.	F.
1931.....	Total.....	63	37.9975	—	—	—
	Between breeds	4	5 4957	1.37	—	2.49
	Within breeds..	59	32.5018	0.55	0.74	—
1930.....	Total.....	53	44.2276	—	—	—
	Between breeds	4	8.9663	2.24	—	<b>3.11</b>
	Within breeds..	49	35.2615	0.72	0.84	—
1929.....	Total.....	49	44.9962	—	—	—
	Between breeds	4	8.7931	2.19	—	2.73
	Within breeds..	45	36.2031	0.80	0.89	—

FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

TABLE XI (cont.).

MEAN DIFFERENCES.

		Hereford.	Ab.-Angus.	Shorthorn.	Afrikaner.
1931.....	Sussex.....	0.12±0.29	0.42±0.27	0.53±0.30	<b>0.77±0.27</b>
	Hereford.....	—	0.30±0.28	0.41±0.32	0.65±0.23
	Ab.-Angus.....	—	—	0.11±0.32	0.35±0.28
	Shorthorn.....	—	—	—	0.24±0.32
		Hereford.	Ab.-Angus.	Shorthorn.	Afrikaner.
1930.....	Sussex.....	0.44±0.40	0.74±0.35	<b>1.11±0.39</b>	<b>1.14±0.35</b>
	Hereford.....	—	0.30±0.38	0.67±0.41	0.70±0.37
	Ab.-Angus.....	—	—	0.37±0.37	0.40±0.33
	Shorthorn.....	—	—	—	0.03±0.36
		Afrikaner.	Shorthorn.	Ab.-Angus.	Sussex.
1929.....	Hereford.....	0.55±0.38	0.80±0.45	0.84±0.36	<b>1.30±0.40</b>
	Afrikaner.....	—	0.25±0.45	0.29±0.37	0.75±0.40
	Shorthorn.....	—	—	0.04±0.43	0.50±0.47
	Ab.-Angus.....	—	—	—	0.46±0.39

TABLE XII.

*Analysis of Variance of Width of Chest.  
Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	38.7956	—	—	—
	Between breeds	4	6.3735	1.59	—	2.90
1930.....	Within breeds..	59	32.4221	0.55	0.73	—
	Total.....	53	33.4253	—	—	—
	Between breeds	4	3.4735	0.87	—	1.42
	Within breeds..	49	29.9518	0.61	0.78	—
1929.....	Total.....	49	37.3113	—	—	—
	Between breeds	4	7.2418	1.81	—	2.71
	Within breeds..	45	30.0695	0.67	0.82	—

MEAN DIFFERENCES.

		Hereford.	Afrikaner.	Ab.-Angus.	Shorthorn.
1931.....	Sussex.....	0.16±0.31	0.54±0.26	0.56±0.26	0.78±0.30
	Hereford.....	—	0.38±0.28	0.40±0.23	0.62±0.31
	Afrikaner.....	—	—	0.02±0.28	0.24±0.31
	Ab.-Angus.....	—	—	—	0.22±0.26
1930.....	Not significant.				
		Afrikaner.	Ab.-Angus.	Shorthorn.	Sussex.
1929.....	Hereford.....	0.64±0.35	0.83±0.33	1.08±0.41	<b>1.16±0.36</b>
	Afrikaner.....	—	0.19±0.33	0.44±0.41	0.52±0.37
	Ab.-Angus.....	—	—	0.25±0.39	0.33±0.35
	Shorthorn.....	—	—	—	0.08±0.43

TABLE XIII.  
*Analysis of Variance of Heart Girth.  
 Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	296 4961	—	—	—
	Between breeds	4	45 9354	11.48	—	2.70
1930.....	Within breeds..	59	250 5604	4.25	2.06	—
	Total.....	53	369.3750	—	—	—
	Between breeds	4	52.7218	13 18	—	2.04
1929.....	Within breeds..	49	316 6532	6.46	2.54	—
	Total.....	49	290.4250	—	—	—
	Between breeds	4	51.6708	12 92	—	2.43
	Within breeds..	45	238 7542	5 31	2 30	—

MEAN DIFFERENCES.

		Sussex.	Ab.-Angus.	Shorthorn.	Afrikaner.
1931.....	Hereford.....	0 23 ± 0 80	1.48 ± 0.79	1.89 ± 0.89	2.05 ± 0.79
	Sussex.....	—	1 25 ± 0 74	1 66 ± 0.85	1.82 ± 0.74
	Ab.-Angus.....	—	—	0 41 ± 0.88	0.57 ± 0.78
	Shorthorn.....	—	—	—	0 16 ± 0 88
1930.....	Not significant.				
		Shorthorn.	Afrikaner.	Ab.-Angus.	Sussex.
1929.....	Hereford.....	1 69 ± 1 17	1 72 ± 0 98	2.42 ± 0.94	2.83 ± 1.03
	Shorthorn.....	—	0.03 ± 1.17	0.73 ± 1 11	1.14 ± 1 21
	Afrikaner.....	—	—	0.70 ± 0.94	1.11 ± 1 03
	Ab.-Angus.....	—	—	—	0.41 ± 0.99

Tables XI to XIII show the differences in chest measurements between breeds. The values of F for depth of chest are significant for all age groups (Table XI). The mean differences in the 1931 group show a significant value only between the Sussex and the Afrikanders in favour of the former breed. In the 1930 group the Sussex are again significantly deeper in the chest than the Afrikanders. In addition, the Sussex also prove significantly deeper than both Aberdeen-Angus and Shorthorns. Other mean differences are not significant. In the 1929 group the only significant differences are those between the Herefords on the one hand and the Aberdeen-Angus and Sussex on the other hand, in favour of the former.

In width of chest the variance between breeds is significant for the 1931 and 1929 groups (Table XII). In the former the mean differences are significant between the Sussex on the one hand and the Afrikanders, Aberdeen-Angus and Shorthorns on the other. In the 1929 group the Herefords are significantly deeper than the Aberdeen-Angus, Shorthorns and Sussex. Other mean differences are not significant.



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In heart girth the variance between breeds is significant in the 1931 and the 1932 groups (Table XIII). On analysis the mean differences show that in the former age group the measurements of the Herefords are significantly greater than those of the Shorthorns and Afrikanders. In the latter age group the Herefords exceed the Aberdeen-Angus and Sussex in a significant degree. The mean differences between other breeds are not significant.

TABLE XIV.  
*Analysis of Variance of Width of Loin.  
Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	21·9678	—	—	—
	Between breeds	4	4·4647	1·12	—	<b>3·76</b>
1930.....	Within breeds..	59	17·5031	0·30	0·54	—
	Total.....	53	26·6309	—	—	—
	Between breeds	4	9·8736	2·47	—	<b>7·22</b>
1929.....	Within breeds..	49	16·7573	0·34	0·58	—
	Total.....	49	27·8263	—	—	—
	Between breeds	4	10·6203	2·65	—	<b>6·94</b>
	Within breeds..	45	17·2060	0·38	0·62	—

## MEAN DIFFERENCES.

		Shorthorn.	Hereford.	Ab.-Angus.	Afrikander.
1931.....	Sussex.....	0·40±0·22	0·54±0·21	0·54±0·20	0·74±0·20
	Shorthorn.....	—	0·14±0·23	0·4±0·23	0·34±0·23
	Hereford.....	—	—	0·00	0·20±0·21
	Ab.-Angus.....	—	—	—	0·20±0·21
1930.....		Ab.-Angus.	Shorthorn.	Hereford.	Afrikander.
	Sussex.....	<b>0·69±0·24</b>	<b>0·72±0·27</b>	<b>0·75±0·28</b>	<b>1·23±0·24</b>
	Shorthorn.....	—	0·03±0·25	0·06±0·26	0·54±0·22
	Hereford.....	—	—	0·03±0·28	0·51±0·25
	Ab.-Angus.....	—	—	—	0·48±0·26
1929.....		Sussex.	Ab.-Angus.	Shorthorn.	Afrikander.
	Hereford.....	0·38±0·28	0·65±0·25	0·84±0·31	<b>1·35±0·26</b>
	Sussex.....	—	0·27±0·27	0·46±0·32	<b>0·97±0·28</b>
	Ab.-Angus.....	—	—	0·19±0·30	<b>0·70±0·25</b>
	Shorthorn.....	—	—	—	0·51±0·31

It will be noted that the breeds do not maintain the same relative positions to each other in the three age groups in respect of chest measurements. In the 1931 and 1930 groups the Herefords and Sussex show the highest values for each of these measurements while

in the 1929 (oldest) group the Sussex have the smallest values for these measurements. A possible explanation for this inconsistency in the behaviour of the Sussex may be sought in a differential growth rate at different ages. This view is supported by the fact that the Sussex showed the second highest average depth of chest and heart girth, in the initial measurements of the 1929 group at the age of 20 months as compared with the highest and second highest averages respectively at the same age in the 1930 and 1931 groups. Similarly in width of chest the average initial measurement of the animals of this breed are midway between those of the other breeds and only slightly below that of the Herefords.

TABLE XV.

*Analysis of Variance of Width at Hooks.*  
*Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	23 8302	—	—	—
	Between breeds	4	8 3190	2 08	—	<b>7.91</b>
1930. . . . .	Within breeds..	59	15.5112	0 25	0 50	—
	Total.....	53	62.9503	—	—	—
	Between breeds	4	21 8905	5.47	—	<b>6.53</b>
1929....	Within breeds..	49	41 0598	0 84	0 91	—
	Total.....	49	54 7513	—	—	—
	Between breeds	4	37 0389	9 26	—	<b>23.52</b>
	Within breeds..	45	17 7124	0 39	0 63	—

## MEAN DIFFERENCES.

		Hereford.	Ab.-Angus.	Shorthorn.	Afrikaner.
1931.....	Sussex.....	0.17±0.19	<b>0.54±0.18</b>	<b>0.64±0.21</b>	<b>1.74±0.18</b>
	Hereford.....	—	0.37±0.19	0.47±0.22	<b>1.57±0.19</b>
	Ab.-Angus.....	—	—	0.10±0.21	<b>1.20±0.19</b>
	Shorthorn.....	—	—	—	<b>1.10±0.21</b>
1930.....		Shorthorn.	Hereford.	Ab.-Angus.	Afrikaner.
	Sussex.....	0.13±0.42	0.14±0.43	0.20±0.37	<b>1.52±0.38</b>
	Shorthorn.....	—	0.01±0.44	0.07±0.40	<b>1.39±0.39</b>
	Hereford.....	—	—	0.06±0.41	<b>1.38±0.40</b>
1929.....	Ab.-Angus.....	—	—	—	<b>1.32±0.35</b>
		Sussex.	Shorthorn.	Ab.-Angus.	Afrikaner.
	Hereford.....	<b>0.81±0.28</b>	<b>0.98±0.32</b>	<b>1.54±0.26</b>	<b>2.41±0.27</b>
	Sussex.....	—	0.17±0.33	<b>0.73±0.27</b>	<b>1.60±0.28</b>
	Shorthorn.....	—	—	0.56±0.30	<b>1.43±0.32</b>
	Ab.-Angus.....	—	—	—	<b>0.87±0.26</b>

TABLE XVI.  
*Analysis of Variance of Width at Thurls.*  
*Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	36.9961	—	—	—
	Between breeds	4	8.8739	2.22	—	<b>4.65</b>
1930.....	Within breeds..	59	28.1222	0.48	0.68	—
	Total.....	53	25.0834	—	—	—
	Between breeds	4	8.9401	2.23	—	<b>6.78</b>
1929.....	Within breeds..	49	16.1433	0.33	0.57	—
	Total.....	49	34.4108	—	—	—
	Between breeds	4	16.3589	4.09	—	<b>10.20</b>
	Within breeds..	45	18.0519	0.40	0.63	—

## MEAN DIFFERENCES.

		Sussex.	Shorthorn.	Ab.-Angus.	Afrikaner.
1931.....	Hereford.....	0.17±0.26	0.50±0.29	0.66±0.26	<b>0.73±0.26</b>
	Sussex.....	—	0.33±0.28	0.49±0.25	0.56±0.25
	Shorthorn.....	—	—	0.16±0.29	0.23±0.29
	Ab.-Angus.....	—	—	—	0.07±0.26
		Shorthorn.	Hereford.	Ab.-Angus.	Afrikaner.
1930.....	Sussex.....	0.10±0.26	0.13±0.27	0.44±0.23	<b>0.91±0.24</b>
	Shorthorn.....	—	0.03±0.28	0.34±0.25	<b>0.81±0.24</b>
	Hereford.....	—	—	0.31±0.26	<b>0.78±0.25</b>
	Ab.-Angus.....	—	—	—	0.47±0.22
		Sussex.	Shorthorn.	Ab.-Angus.	Afrikaner.
1929.....	Hereford.....	<b>1.25±0.28</b>	<b>1.28±0.32</b>	<b>1.36±0.26</b>	<b>1.52±0.28</b>
	Sussex.....	—	0.03±0.33	0.11±0.27	0.27±0.28
	Shorthorn.....	—	—	0.08±0.31	0.24±0.32
	Ab.-Angus.....	—	—	—	0.16±0.26

The measurements of width in the hindquarter are analyzed in Tables XIV to XVI. The following measurements are included: width of loin, width of thurls and width at hooks.

In width of loin (Table XIV) the variance between breeds is highly significant for all age groups. In the 1931 group the Sussex exceed the Hereford, Aberdeen-Angus and Afrikanders in a significant degree. In the 1930 group this breed exceeds all other breeds, the differences being highly significant. In this group the Afrikanders also prove to be significantly narrower in the loin than the Aberdeen-Angus and Shorthorns. Likewise, in the 1929 group the Afrikanders are significantly smaller in the loin than all other breeds except the Shorthorns. In this age group the Herefords also exceed the Aberdeen-Angus and Shorthorns in a significant degree.

In width at hooks the breed differences are also highly significant for all age groups. In each group the Afrikanders show significantly smaller figures than all other breeds. In the 1931 group the figures for the Sussex and Herefords are significantly higher than those of the Shorthorn. In addition, the Sussex exceed the Aberdeen-Angus to a significant extent. In the 1929 group the Herefords and Sussex also exceed the Aberdeen-Angus and in addition the Herefords exceed the Sussex and Shorthorn.

In width at thurls (Table XVI) the Herefords exceed the Afrikanders in all age groups. In the 1931 group they also exceed the Aberdeen-Angus while the Afrikanders are exceeded by the Sussex. In the 1930 group the Afrikanders are exceeded by all groups in a highly significant degree. In the 1929 group the figure for the Herefords is significantly higher than those for all other breeds.

It will be noted that the respective values of F are exceptionally large for all three measurements. This is largely the result of the large mean differences between the Herefords and Sussex at the one extreme and the Afrikanders at the other extreme. The other breeds fall between these extremes and the mean differences between these are small and mostly insignificant.

TABLE XVII.  
*Analysis of Variance of Depth of Flank.  
Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	48 0033	—	—	—
	Between breeds	4	9 7578	2 44	—	<b>3.75</b>
1930.....	Within breeds..	59	38 3355	0.65	0.81	—
	Total.....	53	66 0429	—	—	—
	Between breeds	4	5 8912	1.47	—	1.20
1929.....	Within breeds..	49	60 1517	1.23	1.11	—
	Total.....	49	49 7200	—	—	—
	Between breeds	5	20 7804	5.19	—	<b>7.62</b>
	Within breeds..	45	28.9396	0.65	0.81	—

MEAN DIFFERENCES.

		Afrikander.	Sussex.	Shorthorn.	Ab.-Angus.
1931.....	Hereford.....	0.03±0.31	0.17±0.31	0.86±0.35	0.89±0.31
	Afrikander.....	—	0.14±0.29	0.83±0.34	0.86±0.30
	Sussex.....	—	—	0.69±0.33	0.72±0.29
	Shorthorn.....	—	—	—	0.03±0.34
1930.....	Not significant.				
1929.....		Hereford.	Shorthorn.	Sussex.	Ab.-Angus.
	Afrikander.....	0.41±0.36	<b>1.24±0.41</b>	<b>1.49±0.36</b>	<b>1.55±0.33</b>
	Hereford.....	—	0.83±0.40	<b>1.08±0.36</b>	<b>1.14±0.33</b>
	Shorthorn.....	—	—	0.25±0.42	0.31±0.39
	Sussex.....	—	—	—	0.06±0.33

Referring to Tables XVII and XVIII it is seen that the mean differences between breeds in the 1931 group are significant for depth of flank (Table XVII) but not significant for flank girth (Table XVIII). In the case of the former measurement the Herefords, Afrikanders and Sussex are significantly deeper than the Shorthorns and Aberdeen-Angus.

In the 1930 group the differences are not significant for either measurement. In the 1929 groups the figure for the Herefords is significantly higher than all other breeds in respect of flank girth, and significantly higher than those of the Shorthorns, Sussex and Aberdeen-Angus in respect of depth of flank. The Afrikanders rank second in flank girth and first in depth of flank. In the latter this breed exceeds all breeds, except the Herefords, in a significant degree.

TABLE XVIII.  
*Analysis of Variance of Flank Girth.*  
*Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	414.7874	—	—	—
	Between breeds	4	53.6898	13.42	—	2.19
	Within breeds..	59	361.0976	6.12	2.44	—
1930.....	Total.....	53	476.0787	—	—	—
	Between breeds	4	22.1710	5.54	—	1.67
	Within breeds..	49	453.9077	9.26	3.04	—
1929.....	Total.....	49	190.3800	—	—	—
	Between breeds	4	66.0583	16.51	—	5.98
	Within breeds..	45	124.3217	2.76	1.72	—

MEAN DIFFERENCES.

1931.....	Not significant.				
1930.....	Not significant.				
		Afrikander.	Ab.-Angus.	Shorthorn.	Sussex.
1929.....	Hereford.....	<b>2.14±0.73</b>	<b>2.67±0.70</b>	<b>2.91±0.87</b>	<b>2.91±0.77</b>
	Afrikander.....	—	0.73±0.70	0.77±0.87	0.77±0.77
	Ab.-Angus.....	—	—	0.04±0.83	0.04±0.74
	Shorthorn.....	—	—	—	0.00

In length of pelvis (Table XIX) only the 1929 group shows significant mean differences. The value of  $F=9.575$  is very highly significant but the heterogeneity is largely due to the high figure for the Herefords. The mean differences between this and all other breeds are highly significant. The mean difference between Shorthorns and Sussex is also significant.

The Afrikanders show the smallest values in conformity with the other measurements of the hindquarter.

Table XX shows the analysis of variance of paunch girth. The mean differences between breeds are not significant for the 1931 and 1930 groups. In the 1929 groups only the Herefords exhibit a significantly higher figure than the other breeds except the Sussex.

The relatively large standard deviations indicate that there is great variation within the breeds. It should be noted that in the case of the 1930 group the variance within breeds is greater than that between breeds although not significantly so.

The results obtained by analysis of variance of all body measurements are interpreted to show that the breeds used in this experiment may be variously grouped into two categories depending upon the measurements involved.

TABLE XIX.  
*Analysis of Variance of Length of Pelvis.  
Final Measurements. Half-bred Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	24 9990	—	—	—
	Between breeds	4	2 7824	0.69	—	1.85
	Within breeds..	59	22 2166	0.37	0.61	—
1930.....	Total.....	53	28 3304	—	—	—
	Between breeds	4	3 5803	0.89	—	1.77
	Within breeds..	49	24 7501	0.50	0.71	—
1929.....	Total.....	49	31 0313	—	—	—
	Between breeds	4	14 2792	3.56	—	9.67
	Within breeds..	45	16 7521	0.37	0.60	—

MEAN DIFFERENCES.

1931.....	Not significant.				
1930.....	Not significant.				
		Shorthorn.	Ab.-Angus.	Afrikander.	Sussex.
1929.....	Hereford.....	0.74 ± 0.30	1.21 ± 0.25	1.25 ± 0.26	1.44 ± 0.27
	Shorthorn.....	—	0.47 ± 0.29	0.51 ± 0.30	0.70 ± 0.32
	Ab.-Angus.....	—	—	0.04 ± 0.25	0.23 ± 0.26
	Afrikander.....	—	—	—	0.19 ± 0.27

In height at withers and height over hips the Afrikanders consistently show the highest figures and the Aberdeen-Angus the lowest. The other breeds fall somewhere in between with no consistent order. However, the Shorthorns and Herefords rank closer to the Afrikanders, while the Sussex are more nearly of the same size as the Aberdeen Angus.

TABLE XX.

*Analysis of Variance of Paunch Girth.**Final Measurements of Steers.*

Group.	Source of Variation.	Degrees of Freedom.	Sums of Squares.	Mean Squares.	Standard Deviation.	F.
1931.....	Total.....	63	592.7109	—	—	—
	Between breeds	4	88.2303	22.06	—	—
	Within breeds..	59	504.4606	8.55	2.92	2.58
1930.....	Total.....	53	562.2593	—	—	—
	Between breeds	4	16.7879	4.19	—	—
	Within breeds..	49	545.4714	11.13	3.33	2.65
1929.....	Total.....	49	331.1300	—	—	—
	Between breeds	4	91.1301	22.78	—	—
	Within breeds..	45	240.9990	5.33	2.31	4.27

## MEAN DIFFERENCES.

1931.....	Not significant.				
1930.....	Not significant.				
		Sussex.	Afrikander.	Shorthorn.	Ab.-Angus.
1929.....	Hereford.....	2.01±1.04	2.28±0.98	2.56±1.17	3.88±0.94
	Sussex.....	—	0.27±1.04	0.55±1.22	1.87±1.00
	Afrikander.....	—	—	0.28±1.17	1.60±0.94
	Shorthorn.....	—	—	—	1.32±1.12

In chest measurements the Herefords and Sussex are at the one extreme and the Aberdeen-Angus, Shorthorns and Afrikanders at the other. However, with increasing age the Sussex lag behind and are overtaken by the other breeds, the Herefords maintaining the lead.

In development of hindquarter, the Herefords, Sussex and Shorthorns fall into one class and the Aberdeen-Angus and Afrikanders into the second. In flank measurements the Afrikanders again show high values and are grouped with the Herefords while the other breeds constitute the second group with appreciably lower values.

The tendency to develop a hump coupled with a relatively drooping rump with a consequent prominence in the sacral region, accounts for the high over-all relative height in the Afrikander. Apart from this exception, however, the sum total of all other measurements shows that the Afrikander and Aberdeen-Angus half-breeds are significantly smaller in body build than the Herefords and Sussex. The Shorthorn, which is considered the largest and heaviest among the beef breeds falls between these two groups.

TABLE XXI.  
*Mean Differences in Body Measurements between Males and Females at the age of 14 months.*  
*(Excess in favour of Males.)*  
 Inches.

Group.	Height at Withers.	Height over Hips.	Depth of Chest.	Width of Chest.	Length of Pelvis.	Width of Hooks.	Width of Loin.	Heart of Girth.	Length of Body.
1931 (Measured May, 1933).....	2 23 ( $\pm 1.51$ )	0 97 ( $\pm 1.56$ )	1 07 ( $\pm 0.74$ )	0 58 ( $\pm 0.73$ )	0 65 ( $\pm 0.61$ )	0 83 ( $\pm 0.50$ )	2 22 ( $\pm 0.54$ )	0 23 ( $\pm 2.06$ )	1 11 ( $\pm 1.88$ )
1930 (Measured January, 1933).....	2 83 ( $\pm 1.38$ )	2 93 ( $\pm 1.40$ )	1 10 ( $\pm 0.85$ )	0 06 ( $\pm 0.78$ )	0 56 ( $\pm 0.71$ )	-0 48 ( $\pm 0.91$ )	2 09 ( $\pm 0.58$ )	-0 15 ( $\pm 2.54$ )	1 97 ( $\pm 1.99$ )
1929 (Measured January, 1933).....	4 27 ( $\pm 1.59$ )	4 40 ( $\pm 1.56$ )	2 59 ( $\pm 0.89$ )	1 42 ( $\pm 0.82$ )	1 15 ( $\pm 0.60$ )	0 70 ( $\pm 0.63$ )	7 18 ( $\pm 0.62$ )	0 60 ( $\pm 2.30$ )	4 22 ( $\pm 2.23$ )

TABLE XXII.  
*Analysis of Variance of Height at Withers, Height over Hips, Depth of Chest and Paunch Girth of 1929 Heifers at 44 months of age.*

Body Measurement.	Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Squares.	Standard Deviation.	F.	Standard Deviations for Steers.
Height at Withers.....	Total.....	46	108.0950	—	—	—	—
	Between breeds.....	4	13.3221	3.33	—	—	—
	Within breeds.....	42	94.7729	2.25	1.50	1.21	1.59
Height over hips.....	Total.....	46	13.6878	—	—	—	—
	Between breeds.....	4	3.4196	0.85	—	—	—
	Within breeds.....	42	9.2680	0.22	0.47	3.87	1.56
Depth of Chest.....	Total.....	46	45.4557	—	—	—	—
	Between breeds.....	4	1.8696	0.47	—	—	—
	Within breeds.....	42	43.5861	1.04	1.02	2.22	0.89
Width of chest.....	Total.....	46	64.4894	—	—	—	—
	Between breeds.....	4	7.6151	1.90	—	—	—
	Within breeds.....	42	56.8743	1.35	1.16	1.41	0.82
Paunch girth.....	Total.....	46	530.9401	—	—	—	—
	Between breeds.....	4	87.7670	21.94	—	—	—
	Within breeds.....	42	443.1731	10.55	3.25	2.08	2.31



*Sex Differences.*

In view of the fact that comparable measurements are not available for the females it is not possible to determine accurately sex differences for the various body measurements. However, it is possible to compare the means of the sexes for the dates on which final measurements were taken on the heifers. Such comparison is necessarily unsatisfactory but it will serve to furnish some idea of sex differences in body shape. In the following table mean differences between males and females are given for the most important measurements on a specified date. Standard errors of mean differences have not been calculated but the standard deviations obtained for corresponding final measurements of all steers are indicated in brackets.

It will be seen from Table XXI that the steers exceed the heifers in body measurements in all three age groups. The sex differences are greatest in the following measurements: Height at withers, height over hips, width of loin and body length; and least in length of pelvis, width at hooks and heart girth. Although the differences are relatively small in certain measurements, they are consistent throughout except in the case of width at hooks and heart girth in which the females exceed the males at certain stages of development. It is, of course, a well-known phenomenon in cattle as well as in some other species of farm animals that development of the pelvic region relative to the other body parts is greater in the female than in the male.

It would appear that the females of the same breed are less uniform in shape of body than the males of such breeds but the breed averages do not vary greatly. In order to test this point five measurements for which the 1929 groups of steers show highly significant breed differences were selected and analyzed for the 1929 females. The latter, however, were four months younger than the steers when these measurements were taken.

In the last column of Table XXII the standard deviations of the same measurements for the 1929 steers are indicated. It will be observed that the standard deviations of the chest measurements and paunch girth are greater for the females. That for height at withers is equal to that of the males while in height over hips the females show a comparatively small standard deviation.

In comparing the values of *F* it is observed that in the case of height over hips only is the figure significant as compared with highly significant figures for all five measurements in the case of the steers. Hence it is concluded on the basis of these five body measurements that there is less difference between breeds in the females than there is in the males.

It should be pointed out that the figures used for the females in this analysis are those obtained from the measurements taken in January 1933, at the termination of nine months lactation. If the above body measurements are affected to any extent in the growing animal by lactation, it is possible that the differences between Herefords and Aberdeen-Angus are minimized since a higher percentage of the animals in the former breed passed through a period of lactation. Conversely, the differences between the Afrikanders, on the

one hand, and the Shorthorns and Sussex, on the other, might be exaggerated in view of the higher calving percentage among the former. On the average, however, it is not expected that the means of the females as a whole are greatly influenced by the differential calving rate.

### GENERAL DISCUSSION.

The results obtained from the analysis of both live weights and body measurements tend to show that there are significant differences in growth and development between the breeds studied. The breeds may be divided into two groups, the first comprising Herefords and Sussex showing more rapid growth in all respects, except in over-all height, than the second group consisting of Aberdeen-Angus and Afrikanders. The Shorthorns occupy an intermediate position which is rather surprising and difficult to explain since the purebred Shorthorn is considered one of the most early maturing and largest of all beef breeds. In his study of the weights of bulls, steers and heifers of the various beef breeds, Hammond (1920) found that Shorthorns were appreciably heavier than Herefords, Sussex or Aberdeen-Angus. There is no basis of comparison available for the Afrikander but this breed is generally more rangy in conformation than any of the British beef breeds.

There is no satisfactory method for expressing relative size in exact mathematical terms. Yapp's (1923) dimension-weight index, when applied to the present data, gives the following figures:—

Age Group.	1929.	1930.	1931.
Sussex half-breeds.....	4.962	4.988	5.467
Hereford half-breeds.....	5.038	5.178	5.150
Aberdeen-Angus half-breeds.....	5.132	5.046	5.581
Shorthorn half-breeds.....	5.310	5.447	5.583
Afrikander half-breeds.....	5.330	5.583	5.540

These figures are in substantial agreement with the above classification of the breeds. It should be noted that the dimension-weight index diminishes with age as pointed out by Yapp. The index of the Shorthorns at any age is practically the same as that for the Afrikanders. This shows that the former were comparatively as rangy as the Afrikanders. What is the explanation for this inconsistency in the development of the Shorthorn half-breeds? It is difficult to estimate the relative importance of environmental and genetic factors involved in the growth of the animal. In this case all breeds were exposed to more or less similar environmental conditions. Under natural range conditions the cattle are dependent upon the natural pasturage for their nutritional requirements throughout the year. The seasonal effects upon the development of these animals has been clearly demonstrated in preceding pages. The supply of natural forage is intimately associated with season and the question of seasonal influence on growth and development resolves itself largely into one of nutrition. Other seasonal factors, such as temperature and humidity, undoubtedly also exert some influence but the nutritional factor appears to be the most important.

No explanation is offered for the peculiar behaviour of the Shorthorn half-breds. It is possible, of course, that the pure-bred bulls used were exceptionally poor specimens of the breed although precautions were taken to ensure that they should be representative. Alternatively, the Shorthorn may not be so well adapted as the other breeds to the severe climatic conditions, including poor feed supply, obtaining under these conditions. It is believed, however, that an explanation must be sought in a combination of these factors. The bulls when purchased as young animals, showed comparatively good type but they became more rangy at maturity. The question as to whether they produced rangy offspring because they were of a more rangy constitution genetically than the average of the breed or whether both the bulls and their offspring developed ranginess consequent upon conditions of severe climate or inadequate nutrition, remains unanswered. Attention is drawn to the fact that the dimension-weight index of the Shorthorns in the youngest (1931) age group is approximately the same as those of all other breeds, with the exception of the Hereford, but shows relatively little change with increasing age. For purposes of comparison the dimension-weight index of the dams of the 1929 group is given below:—

	<i>Dimension-Weight Index.</i>
Dams of Sussex half-breds ... ..	5.011
„ „ Hereford half-breds ... ..	5.230
„ „ Aberdeen-Angus half-breds ... ..	5.202
„ „ Shorthorn half-breds ... ..	4.978
„ „ Afrikander half-breds ... ..	5.282

It will be seen that the figure for the dams of the Shorthorns is the lowest, indicating the least ranginess, although the differences between all groups are relatively unimportant.

There is no satisfactory method of measuring the relative “prepotency” of the sires of different breeds used. The limited number of sires (two of each breed) and the fact that no breeding records of individual bulls of the same breed were kept preclude the use of sire-offspring correlations. Only well defined breed characteristics such as the colour pattern of the Hereford, the polled condition of the Angus and the distinguishing features of the Afrikander, viz., sleekness of coat, loose skin and a tendency to develop a hump are clearly shown in the offspring. These traits may be easily observed in the illustrations of half-bred calves of different breeding shown in Appendix figures 33 to 37. More or less typical half-bred cows of the different breeds are illustrated in Figures 38 to 43.

In the discussion on the comparative weights of dams and daughters it was observed that the difference in average weights between dams and daughters was exceedingly small and the question was raised whether any improvement has been brought about by the use of pure-bred bulls on the nondescript cows. The situation is identical in regard to all body measurements. The daughters were only 48 months old at the time the final weights were taken and consequently had not reached maximum development in body size but it is doubtful if they would exceed their dams in weight to any

marked extent even at maturity. Hansen (1925) states that the Black and White Lowland cattle of Eastern Prussia do not show any appreciable growth in weight after 4 years of age but Hammond (1920) states: "The continental breeds on the whole show slightly greater early maturity ratios than those of the British breeds—for bulls: Simmentaler 86·2 per cent., Black and White Lowland 88·4 per cent. and Allgau 80·9 per cent."

In the crossing of two distinct races or breeds of animals the offspring usually exhibit increased size and vigour over the parental types due to the phenomenon of heterosis, especially where there has been some inbreeding in the parental strains. Visual appraisal of the animals failed to indicate marked heterosis in these half-breeds, particularly in the case of the females. This may be due to the fact that their dams were not representative of a distinct type since blood of the European and British breeds have been introduced into the native stock at various times. Better results might be expected if cows of the distinct native types had been used as some of these types have been bred along the same lines for many generations and inbreeding in various degrees commonly occurred—usually unintentionally.

The inheritance in animals is controlled by genes which may be either specific or general in their effects. It would appear from data on the Afrikaner that height over hips may be controlled to some extent at least, by specific genes. The same applies to height over withers. The possibility of the existence of "group" factors for hip height, wither height and hump in the Afrikaner, must also be considered. From his analysis of growth data on the rabbit, Wright (1932) concludes that the influence of general size factors preponderates but he found indications of such group factors affecting head growth apart from general size, others for fore-limbs and hind-limbs collectively, and a third group for the hind-limbs separately. Gregory (1933) from his studies on the inheritance of size in dairy cattle found indications of at least three different genetic compositions involved in height.

The following dam-offspring correlation coefficients were obtained for live weight and certain body measurements specified:—

	<i>Dam-Offspring Correlations.</i>
Height at withers ... ..	·38
Width at thurls ... ..	·32
Width at hooks ... ..	·31
Live weight ... ..	·27
Body length ... ..	·24
Heart girth ... ..	·18
Width of chest ... ..	·13
Depth of chest ... ..	·10

The above correlations are a weighted average of the correlations within the various breeds computed by the method of analysis of covariance after Fisher (1933), by means of which the necessary corrections have been made for group (breed) heterogeneity.

It will be noticed that the figures for live weight, height at withers, width at thurls, width at hooks and body length are significant. That for heart girth borders on significance while those for width and depth of chest respectively are not significant. It is possible by the application of Wrigth's (1921) formula for the parent-offspring correlation to estimate the proportion ( $h^2$ ) of the variance within each group due to heredity assuming that there is no dominance or interaction (nicking), mating being at random within groups as in the present case.

$$r_{po} = 1/2 h^2$$

then  $h^2$  has the following values for the different traits:—

	<i>Variance.</i>	
	<i>Hereditary.</i>	<i>Environmental.</i>
Height at withers ... ..	·76	·24
Width at thurls ... ..	·64	·36
Width at hooks ... ..	·62	·38
Live weight ... ..	·54	·46
Body length ... ..	·48	·52
Heart girth ... ..	·36	·64
Width of chest ... ..	·26	·74
Depth of chest ... ..	·20	·80

The figures in the last column indicate the proportion of the variance in different body traits, within the animals under the conditions of this experiment, due to factors other than genetic ones which can be expressed as combining additively.

It will be observed that a high proportion of the variance found in height over withers is hereditary and this measurement is apparently least affected by external agencies. Likewise in width at thurls and width at hooks more than 60 per cent. of the variance is genetic. In body length more than half the variance is shown as due to environmental factors. However, this measurement is influenced to a considerable extent by the position in which the animal stands at the time of measuring and is probably not very accurate. Random inaccuracies would increase the proportion of variance listed in the above correlation as environmental.

In the case of the chest measurements it would appear that the variance is but slightly hereditary. The extremely small correlation coefficient for depth of chest is difficult to explain. While heart girth and width of chest are known to be influenced to a considerable extent by environmental factors one would not expect the same situation in depth of chest which depends mostly upon dimensions of bones.

In live weight approximately half of the variance in these animals is due to causes other than genetic in origin. This varying degree of susceptibility to environmental influences explains why such factors as seasonal fluctuations and feed supply exert a noticeably greater influence on development in body weight and certain body measurements than on others in the present study as shown by the growth curves in figures 7 and 8.

In a study of the genetic constitution of pure-bred Jersey cattle in the United States, Gowen (1933) found great variability in the relation between dam's type and daughters' type within certain herds but he concludes that inheritance accounts for most of the variation in the size of the animals, such environmental differences as do exist playing but little part in the ultimate constitution of the animals. It will be readily understood, of course, that high grade dairy cattle are usually kept under uniformly good conditions and that they are not likely to have been subjected to as extreme a range of environmental conditions as these range cattle in the Northern Transvaal. Furthermore it is quite probable that a fairly high degree of assortative mating genetically has occurred in the Jersey breed.

The dam-offspring correlations in the present study clearly show the varying degree of susceptibility of various body traits to environmental influences. This leads to the conclusion that no sharp and universally valid line can be drawn between environmental and genetic factors in their relative importance in growth and development. Both have a physiological basis. In the words of Wright (1933) it is rather common "to treat environmental influences as requiring detailed physiological analysis but to assume that assignment of an effect to heredity ends the matter. The genes carried in the nuclei of the cells can only control growth, or any other character, through physiological channels, starting from primary effects on cell metabolism". There is thus clearly plenty of room for control of growth characters both by breeding and management.

### SUMMARY.

Data are presented on the growth of range cattle over a period of four years in a semi-arid region of South Africa. A total of 176 half-bred animals of five different breeds are included in this study.

The data consist of live weights taken at bi-monthly intervals of all animals, and linear body measurements taken at bi-monthly intervals for a period of 28 months for three different age groups.

Growth in weight is strictly seasonal from weaning to maturity. These seasonal fluctuations are shown to be closely allied to monthly rainfall. The bulk of the annual precipitation occurs from November to February and the period of greatest relative growth in weight lags approximately three months behind the period of heaviest precipitation.

The greatest increase in body weight occurs from January to March and the periods of smallest increase or greatest loss in weight occur from July to September. Body measurements are also influenced by seasonal changes but to a lesser extent than body weight. The following measurements are least affected: height at withers, height over hips, body length, depth of chest and depth of flank. The measurements markedly influenced by season of the year are length of pelvis, width at hooks, width at thurls, width of loin and width of chest.

Significant differences between the sexes exist from birth, males being heavier than females.

Sex differences in body measurements are not so marked, especially in the earlier age groups. When maturity is approached the steers exceed the heifers to a marked degree in all body measurements except those of the pelvic region.

The breeds may be divided into two classes in respect to weight and size, Herefords and Sussex comprising one class and Aberdeen-Angus and Afrikaner constituting another. The animals of the first class exceed those of the second class in weight and most body measurements. The Shorthorns occupy an intermediate position and the peculiar behaviour of this breed is discussed.

The Afrikanders proved to have greater wither height and hip height than all other breeds.

The relative importance of genetic and environmental factors on the growth of the animals is discussed.

It is shown that the variance in height at withers in this population is approximately three-fourths genetic and one-fourth environmental in origin. In width at thurls and width at hooks more than 60 per cent of the variance is genetic. The variance in body length and live weight is accounted for equally by genetic and environmental factors. In all chest measurements the variance appears to be only slightly hereditary.

It is shown that the factor of nutrition plays a very important rôle in the growth of range cattle under the conditions of this experiment. It is not possible to determine from this study whether permanent stunting results from inadequate nutrition but it is concluded that growth is retarded and maturity delayed.

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## APPENDIX.

TABLE XXIII.  
Average Live Weights in Pounds 1931 Half-breds.

Breed of Sire.....	Hereford.		Shorthorn.		Sussex.		Afrikaner.		Ab.-Angus.	
Sex.....	Steers.	Heifers.	Steers.	Heifers.	Steers.	Heifers.	Steers.	Heifers.	Steers.	Heifers.
No. of Animals.....	12	14	9	6	15	10	14	14	14	9
Average Birth Date.....	5 June.	31 May.	26 May.	6 June.	5 June.	13 June.	8 June.	17 June.	31 May.	2 June.
Average Birth Weight.....	72	66	66	68	72	61	62	57	56	58
1931.										
Weight on—										
22 July.....	143	133	137	134	139	123	134	113	127	136
18 September.....	212	188	207	197	217	192	198	181	198	207
19 November.....	306	280	283	286	313	280	287	262	284	290
1932.										
20 January.....	442	399	413	405	419	380	409	372	373	375
18 March.....	496	438	450	443	501	456	465	412	435	433
18 May.....	526	479	489	483	541	495	502	437	470	462
17 July.....	547	493	505	504	559	511	519	451	484	478
17 September.....	572	499	526	507	580	508	529	448	502	486
23 November.....	545	494	498	498	557	500	507	445	485	473
1933.										
20 January.....	618	555	571	561	627	566	569	499	554	535
17 March.....	692	612	629	618	699	632	652	538	616	594
20 April.....	765	690	705	683	765	683	710	626	689	659
15 May.....	789	709	714	710	779	719	727	631	707	684
15 June.....	751	703	690	699	756	720	694	634	696	678
18 July.....	788	711	721	712	791	731	739	645	721	691
16 August.....	804	685	723	690	798	706	746	625	724	670

TABLE XXIV.  
Average Live Weights in Pounds 1930 Half-breds.

Breed of Sire .....	Hereford.		Shorthorn.		Sussex.		Afrikaner.		Ab.-Angus.	
	Steers. 8 14 May. 66	Heifers. 12 9 May. 65	Steers. 9 11 May. 69	Heifers. 8 3 May. 59	Steers. 10 21 May. 72	Heifers. 7 11 May. 69	Steers. 14 18 May. 65	Heifers. 16 10 May. 61	Steers. 13 8 May. 61	Heifers. 10 5 May. 56
Sex .....										
No. of Animals .....										
Average Birth Date .....										
Average Birth Weight .....										
1931.										
Weight on—										
16 January .....	362	367	411	370	401	397	339	368	393	366
16 March .....	455	421	464	421	462	450	403	416	442	412
18 May .....	510	492	505	471	515	507	469	478	497	466
17 July .....	501	501	493	475	520	504	470	474	493	464
16 September .....	558	547	567	530	585	572	519	525	549	519
16 November .....	601	570	597	544	614	587	555	546	600	541
1932.										
13 January .....	716	694	713	664	742	703	664	652	694	615
16 March .....	730	709	732	690	763	723	708	677	715	682
16 May .....	810	780	820	745	856	791	755	746	801	734
15 July .....	833	813	825	777	868	831	789	775	816	754
16 September .....	835	816	812	778	853	841	781	781	798	758
21 November .....	795	779	803	769	848	840	770	771	792	741
1933.										
19 January .....	893	837	884	842	937	900	832	831	874	795
15 March .....	960	865	943	864	1,003	908	912	846	929	795
20 April .....	1,021	910	1,004	834	1,059	908	907	864	991	837
15 May .....	1,027	924	1,006	836	1,075	923	972	881	1,001	855
14 June .....	1,002	908	967	812	1,050	862	946	862	964	834
18 July .....	984	921	959	794	1,018	864	926	860	953	847
16 August .....	1,015	894	994	755	1,059	843	948	836	984	820

TABLE XXV.  
Average Live Weight in Pounds 1929 Half-breds.

Breed of Sire.....	Hereford.		Shorthorn.		Sussex.		Afrikaner.		Ab.-Angus.	
	Steers. 11 23 May. 67	Heifers. 12 1 June. 59	Steers. 6 19 May. 69	Heifers. 10 18 May. 57	Steers. 9 20 May. 68	Heifers. 8 13 May. 71	Steers. 12 27 May. 62	Heifers. 10 25 May. 56	Steers. 13 11 June. 56	Heifers. 8 23 May. 56
Sex.....										
No. of Animals.....										
Average Birth Date.....										
Average Birth weight.....										
1930.										
Weight on—										
13 January.....	406	361	387	368	377	413	382	341	346	340
11 March.....	480	405	456	412	443	473	457	405	411	406
14 May.....	604	523	579	535	550	593	565	502	512	514
14 July.....	607	515	563	518	556	594	559	492	513	510
13 September.....	635	551	586	541	588	623	583	515	533	532
14 November.....	635	542	575	547	590	616	562	512	521	522
1931.										
16 January.....	691	607	642	599	654	680	651	510	601	582
16 March.....	767	659	713	656	725	725	720	626	674	649
18 May.....	833	703	764	710	762	774	774	680	724	682
17 July.....	844	706	765	728	763	738	777	684	723	677
16 September.....	880	726	795	720	783	758	805	705	763	693
16 November.....	857	732	805	716	800	755	812	715	775	693
1932.										
18 January.....	1,009	844	954	829	922	856	933	824	897	782
16 March.....	998	831	957	831	928	873	943	839	908	765
16 May.....	1,125	859	1,059	865	1,022	924	1,033	842	1,004	821
15 July.....	1,147	847	1,057	871	1,034	914	1,052	820	1,019	836
16 September.....	1,133	813	1,019	854	1,001	897	1,028	800	985	835
19 November.....	1,074	735	966	789	963	824	998	732	961	768
1933.										
18 January.....	1,163	802	1,067	876	1,050	917	1,072	794	1,048	834
16 March.....	1,227	829	1,118	928	1,097	962	1,121	848	1,091	870
18 April.....	1,289	884	1,173	956	1,147	1,017	1,181	886	1,149	911
15 May.....	1,312	905	1,187	953	1,159	1,037	1,197	900	1,154	905
14 June.....	1,257	869	1,139	884	1,117	1,018	1,148	885	1,115	862
18 July.....	1,257	864	1,143	894	1,123	1,023	1,158	895	1,129	869
16 August.....	1,270	864	1,179	866	1,105	1,000	1,153	896	1,119	858
Average weights of dams.....	940	922	972	846	962	956	870	844	835	892

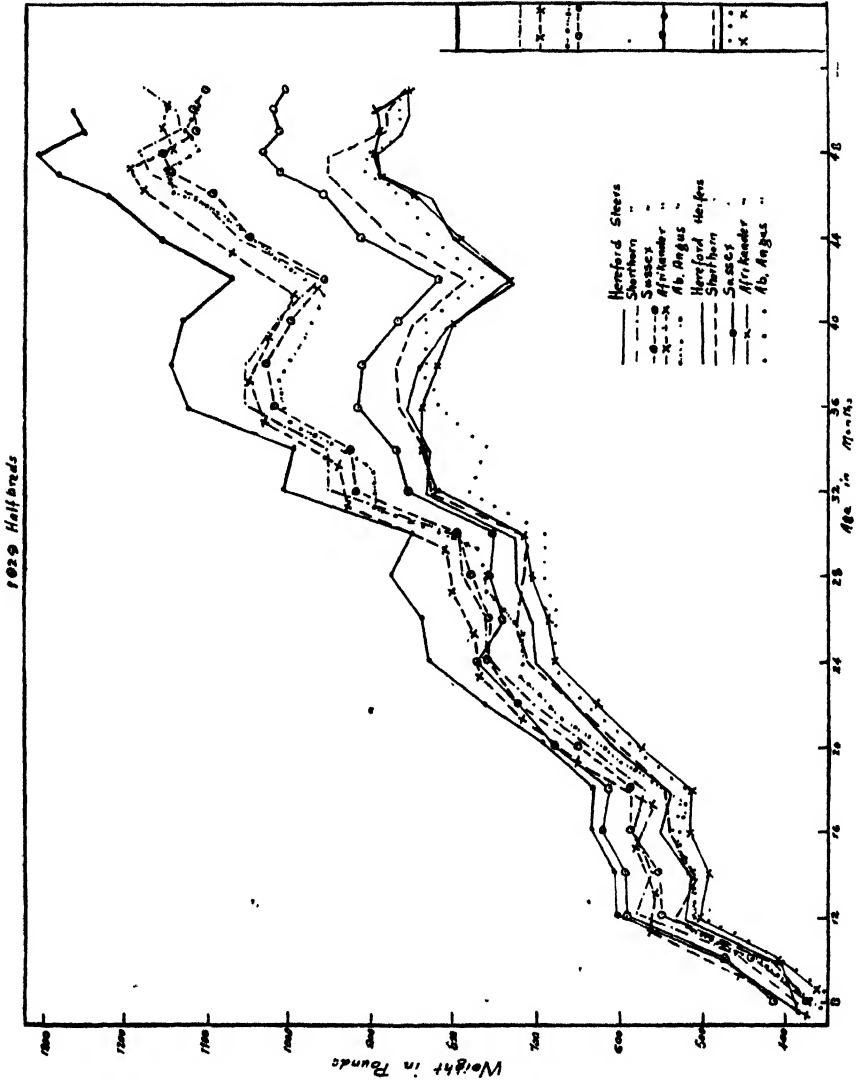


Fig. 9.—Average growth curves of steers and heifers. Live weight.

1930 Halfbreeds

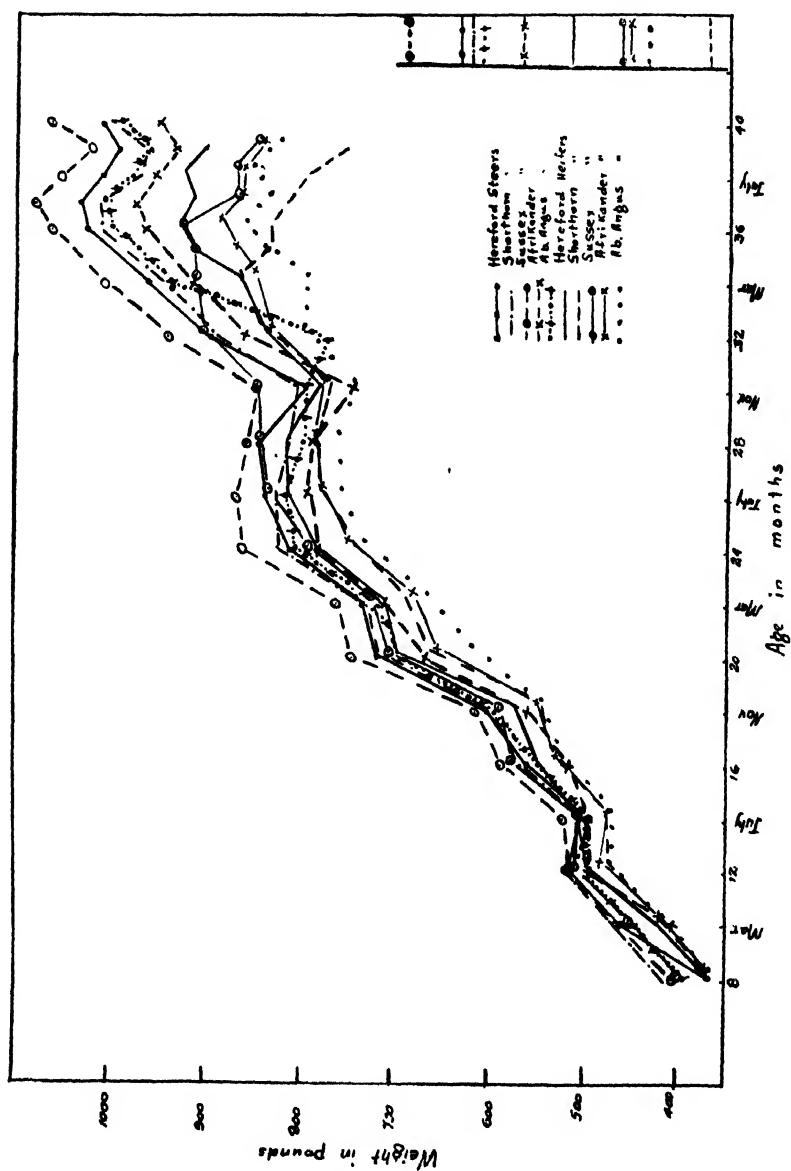


Fig. 10.—Average growth curves of steers and heifers. Live weight.

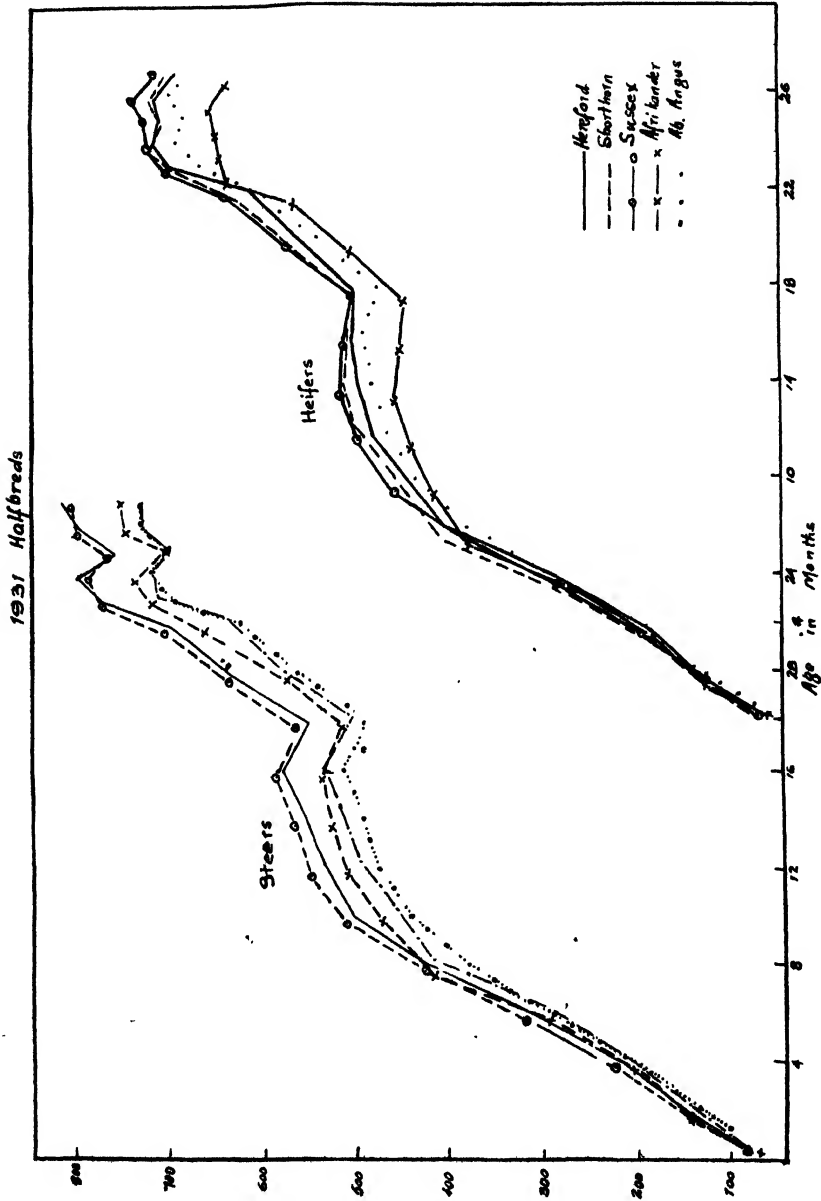


Fig. 11.—Average growth curves of steers and heifers. Live weight.

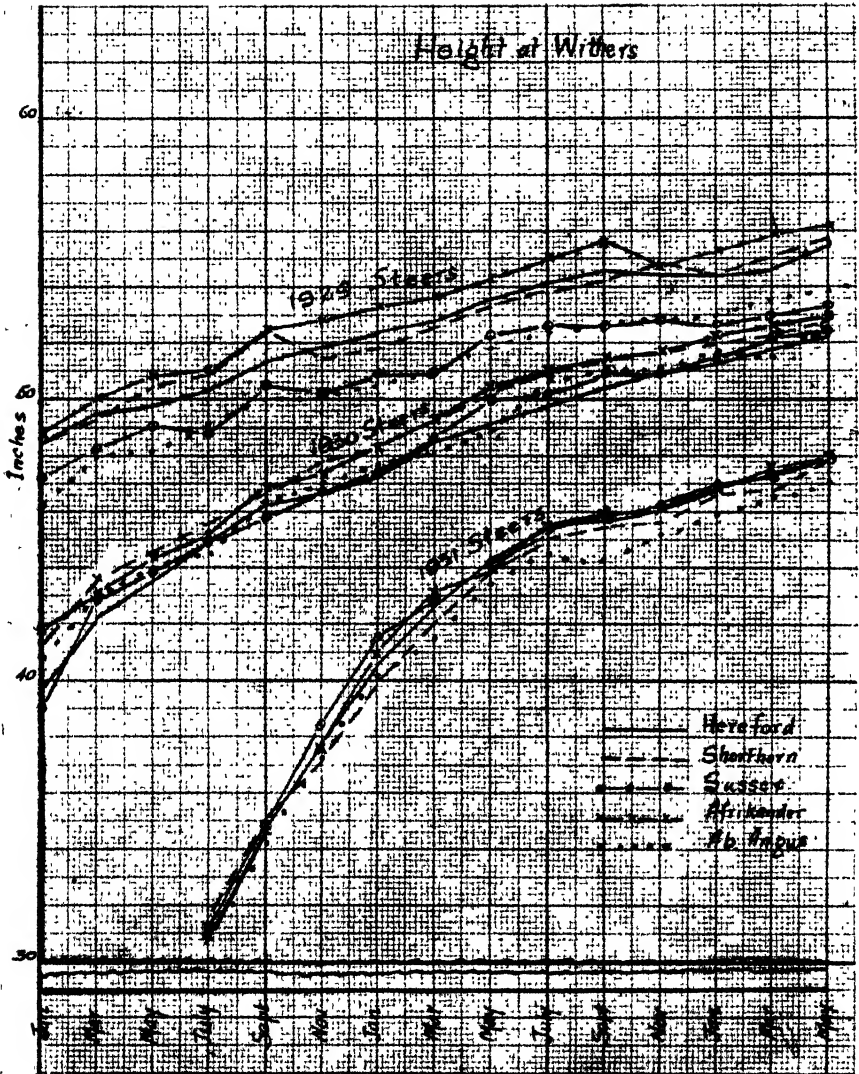


Fig. 12.—Average growth curves of steers. Height at withers.



FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

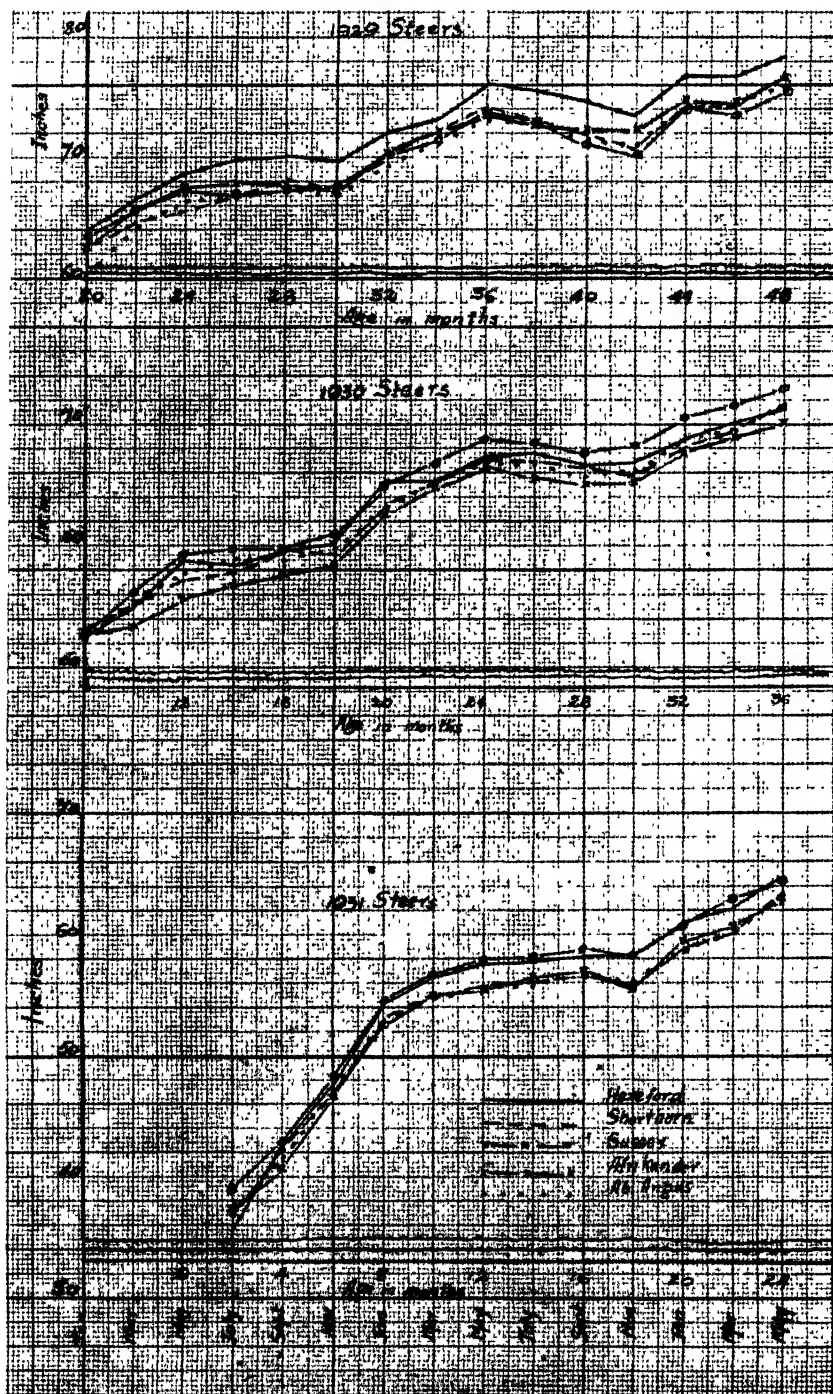


Fig. 13.—Average growth curves of steers. Heart girth.

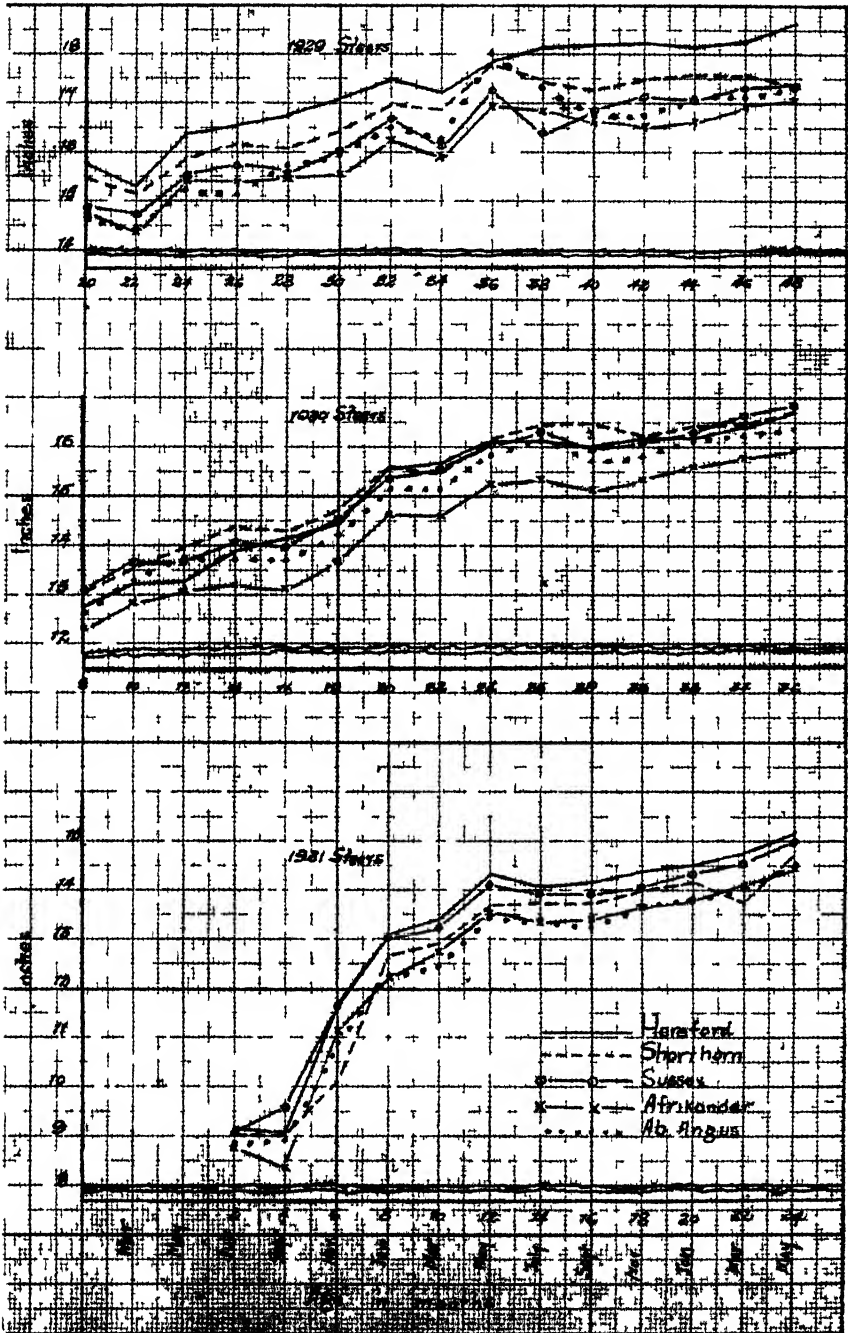


Fig 14 —Average growth curves of steers Width at thurls.

FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

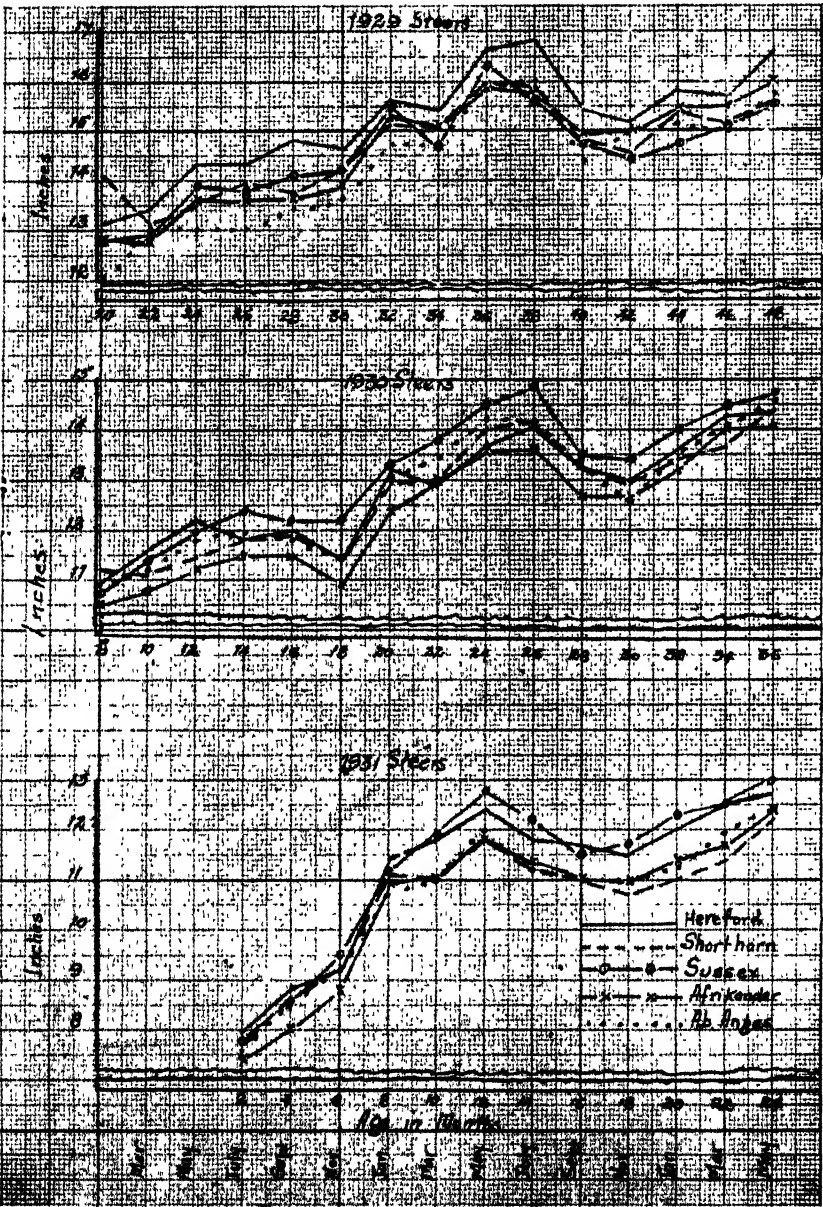


Fig. 15.—Average growth curves of steers. Width of chest.

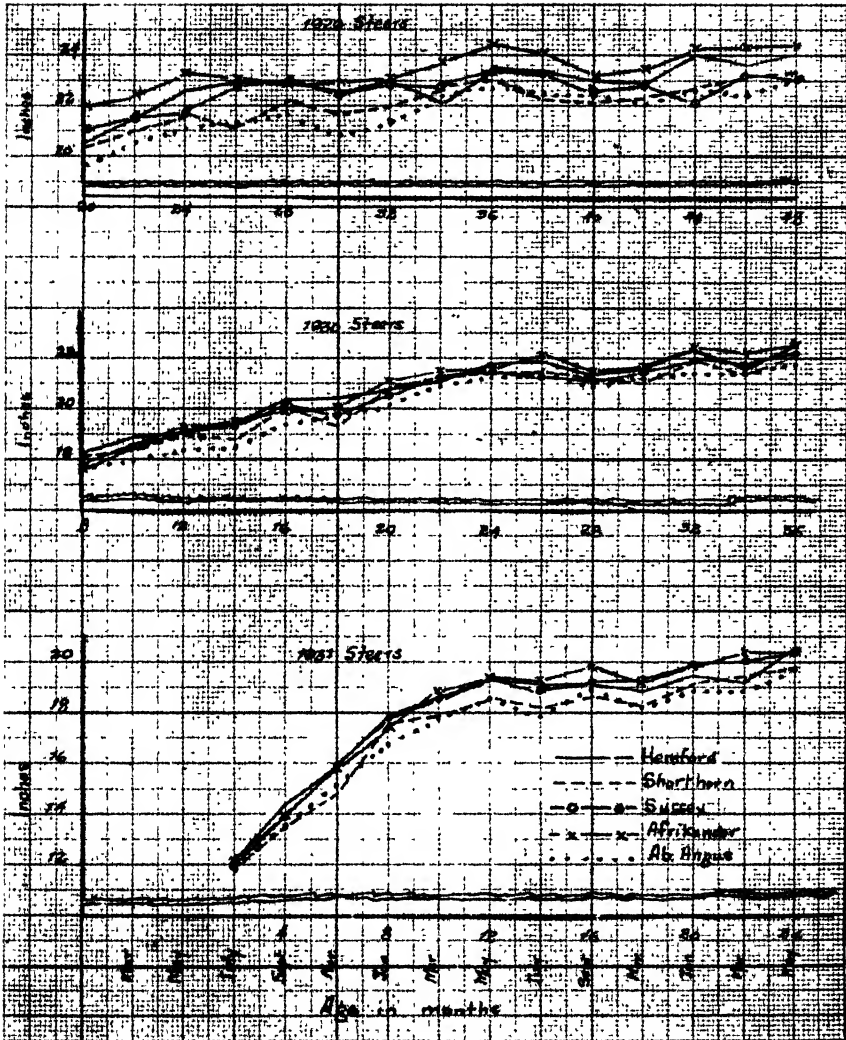


Fig. 16.—Average growth curves of steers: Depth of flank.

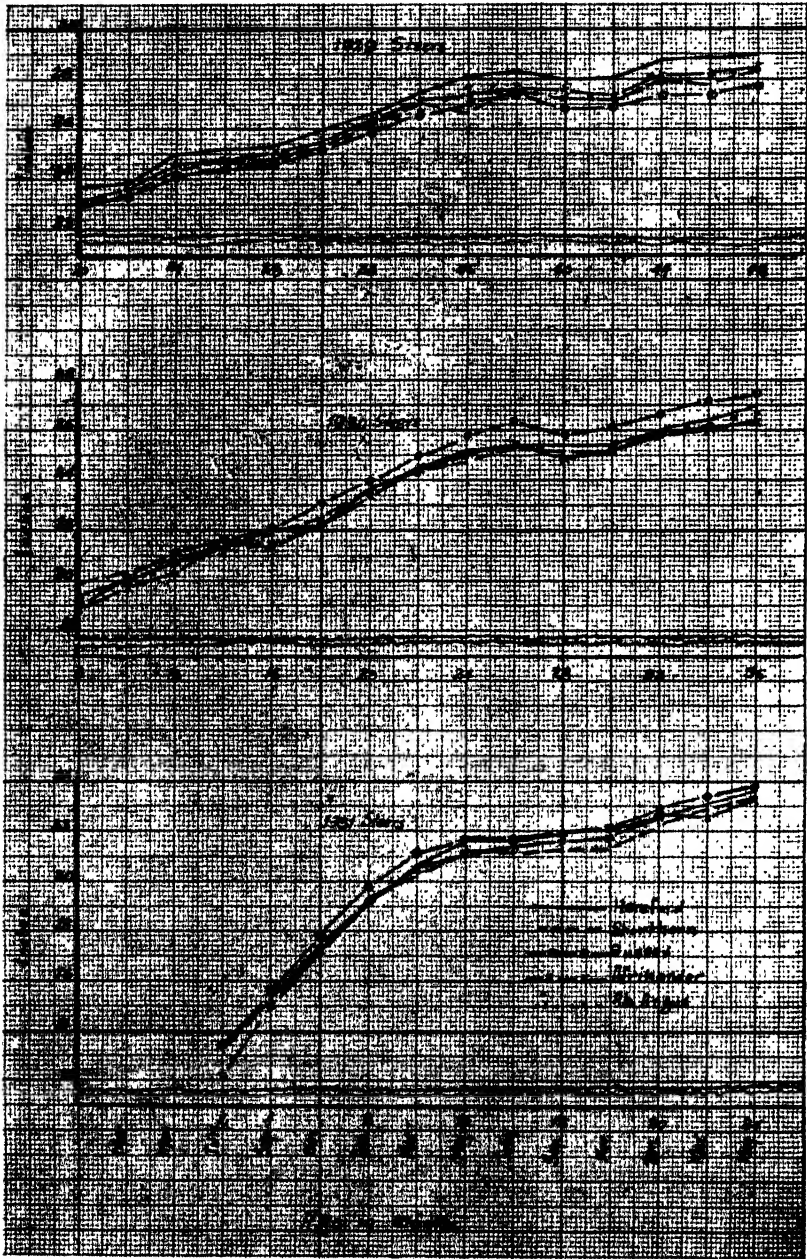


Fig. 17.—Average growth curves of steers. Depth of chest.

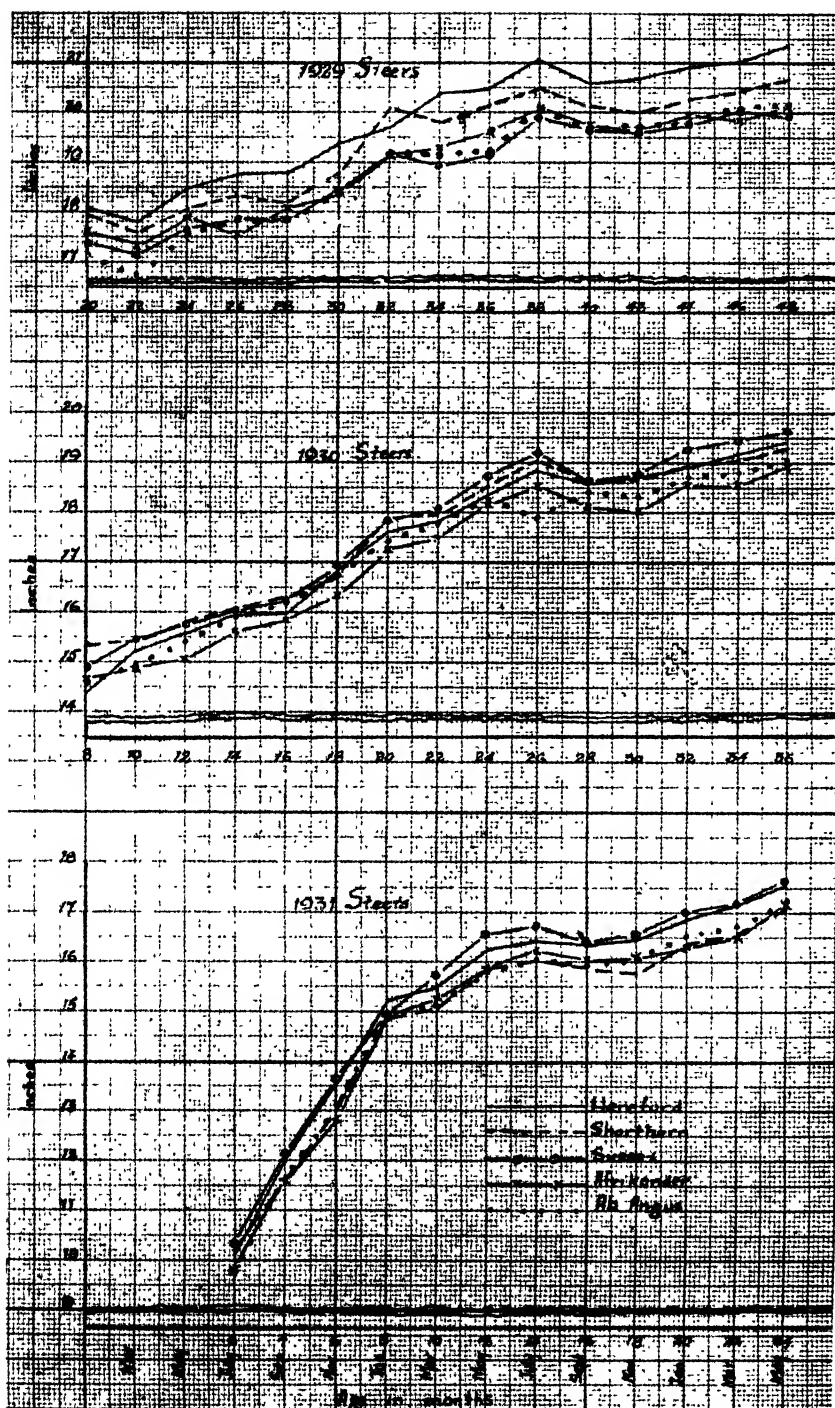


Fig. 18.—Average growth curves of steers. Length of pelvis.



FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

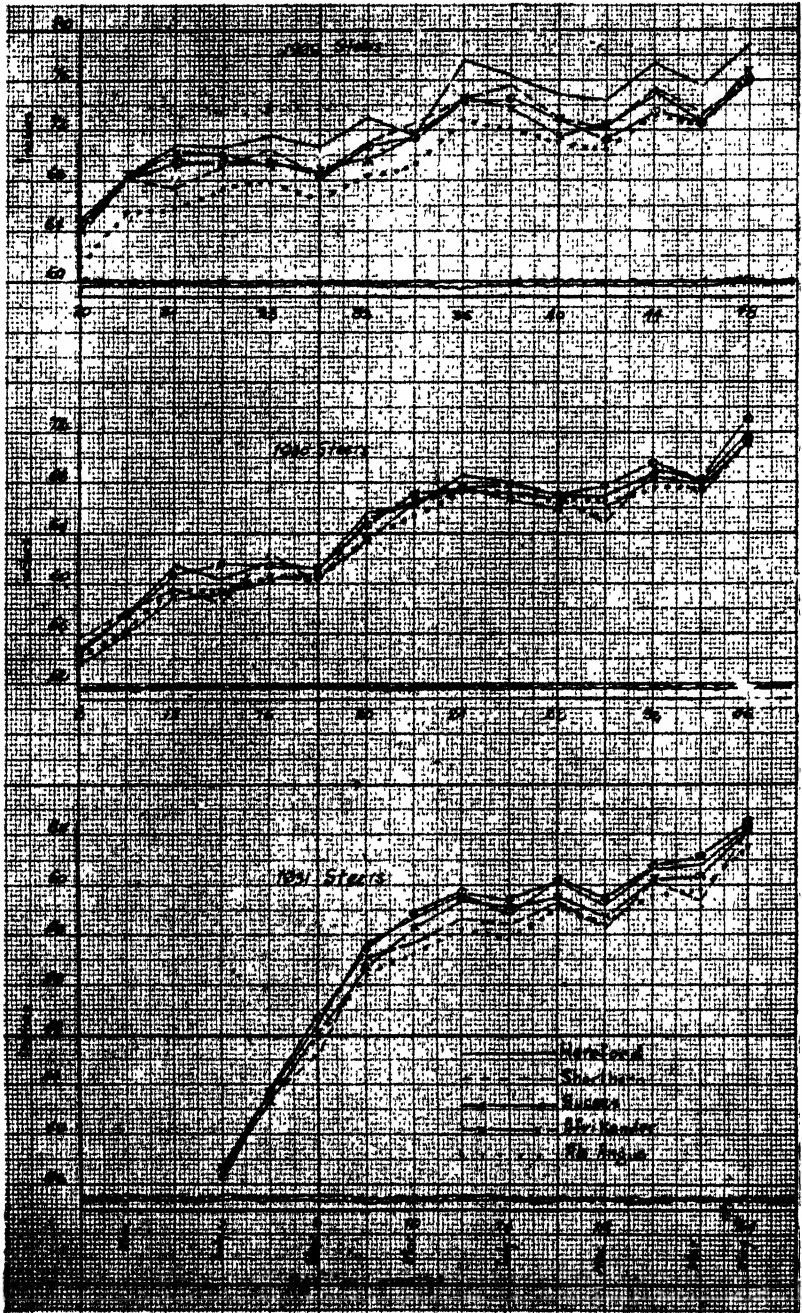


Fig. 19.—Average growth curves of steers. Flank girth.

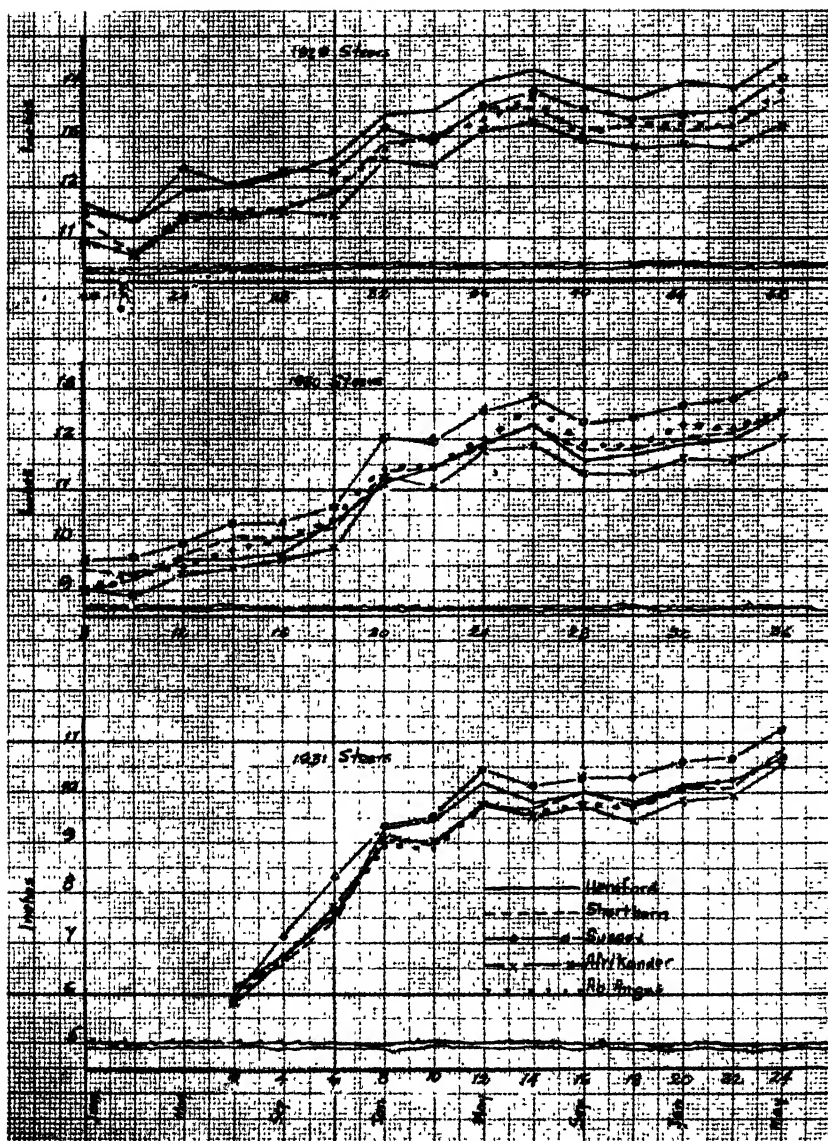


Fig. 20.—Average growth curves of steers. Width of loin.



FACTORS AFFECTING GROWTH OF RANGE CATTLE IN SEMI-ARID REGIONS.

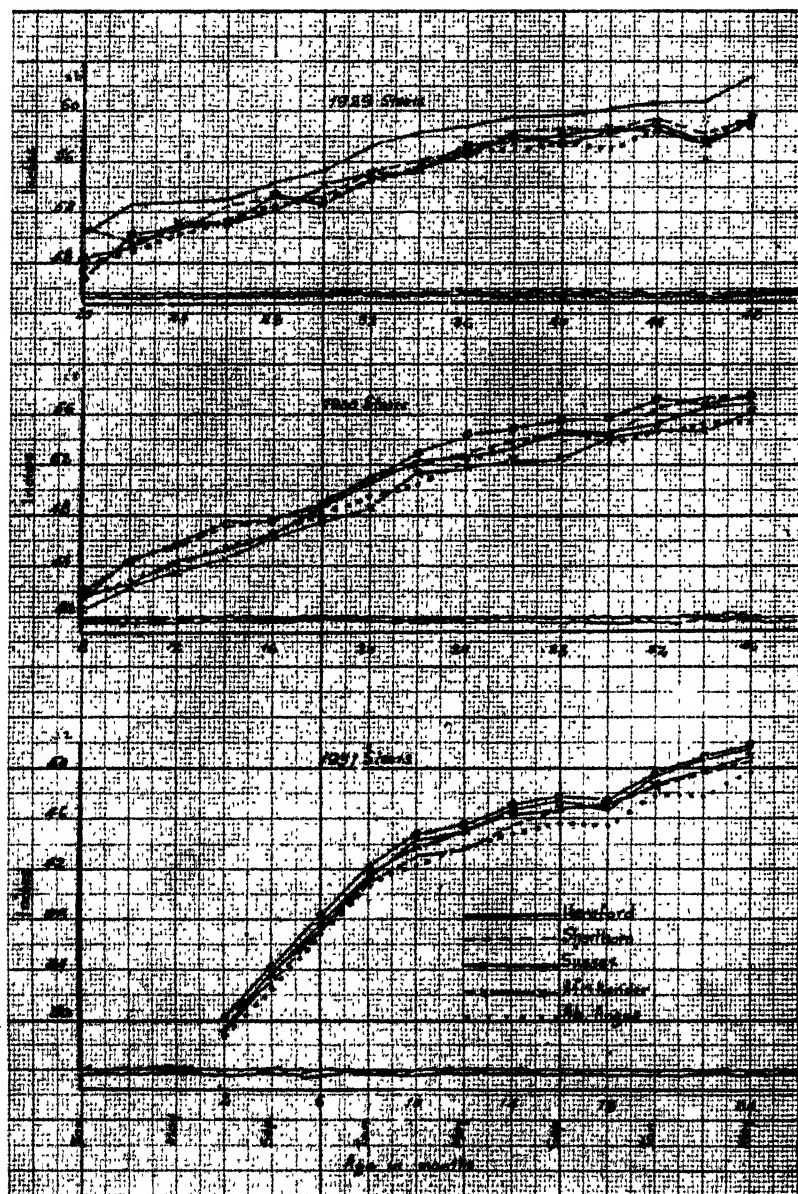


Fig. 21.—Average growth curves of steers. Length of body.

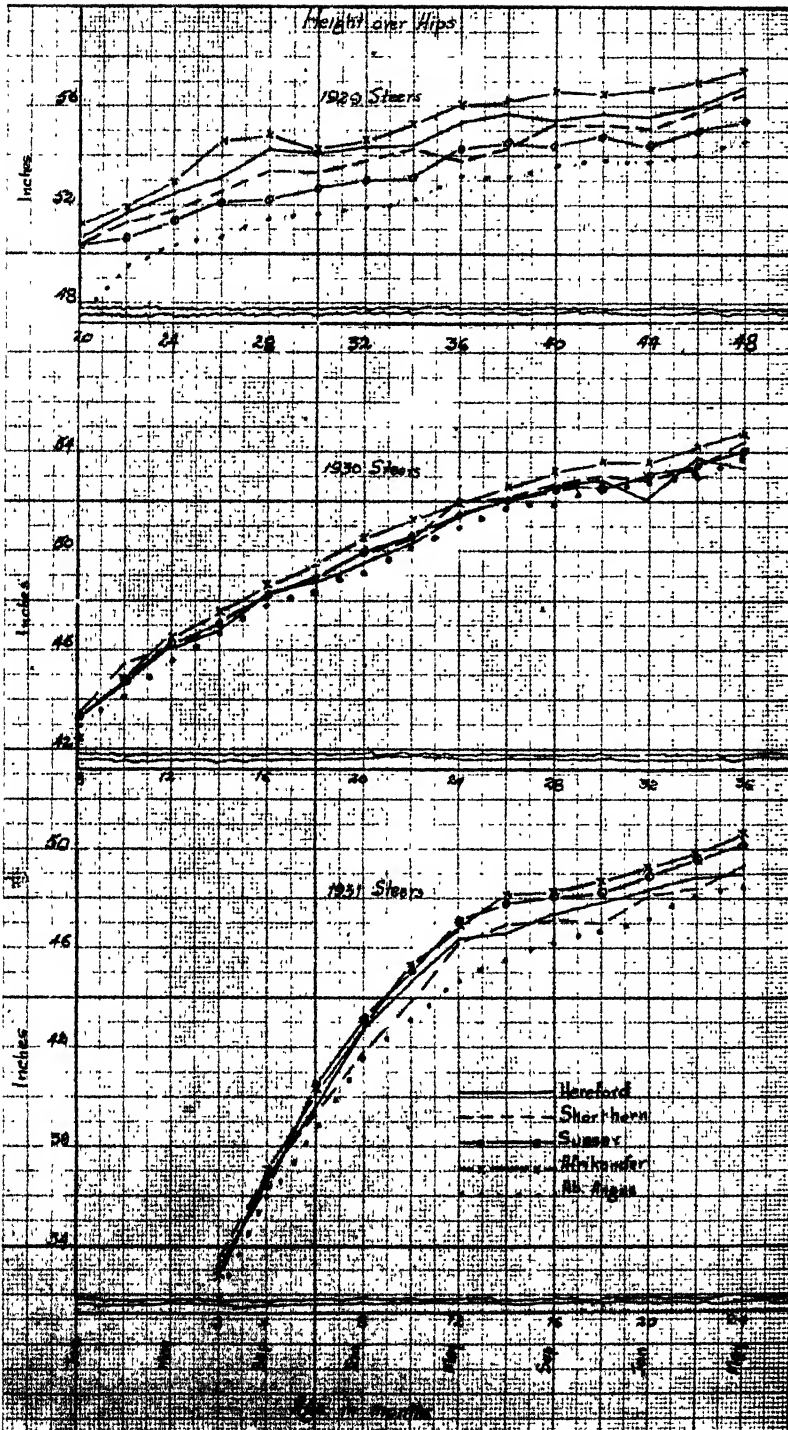


Fig. 22.—Average growth curves of steers. Height over hips.

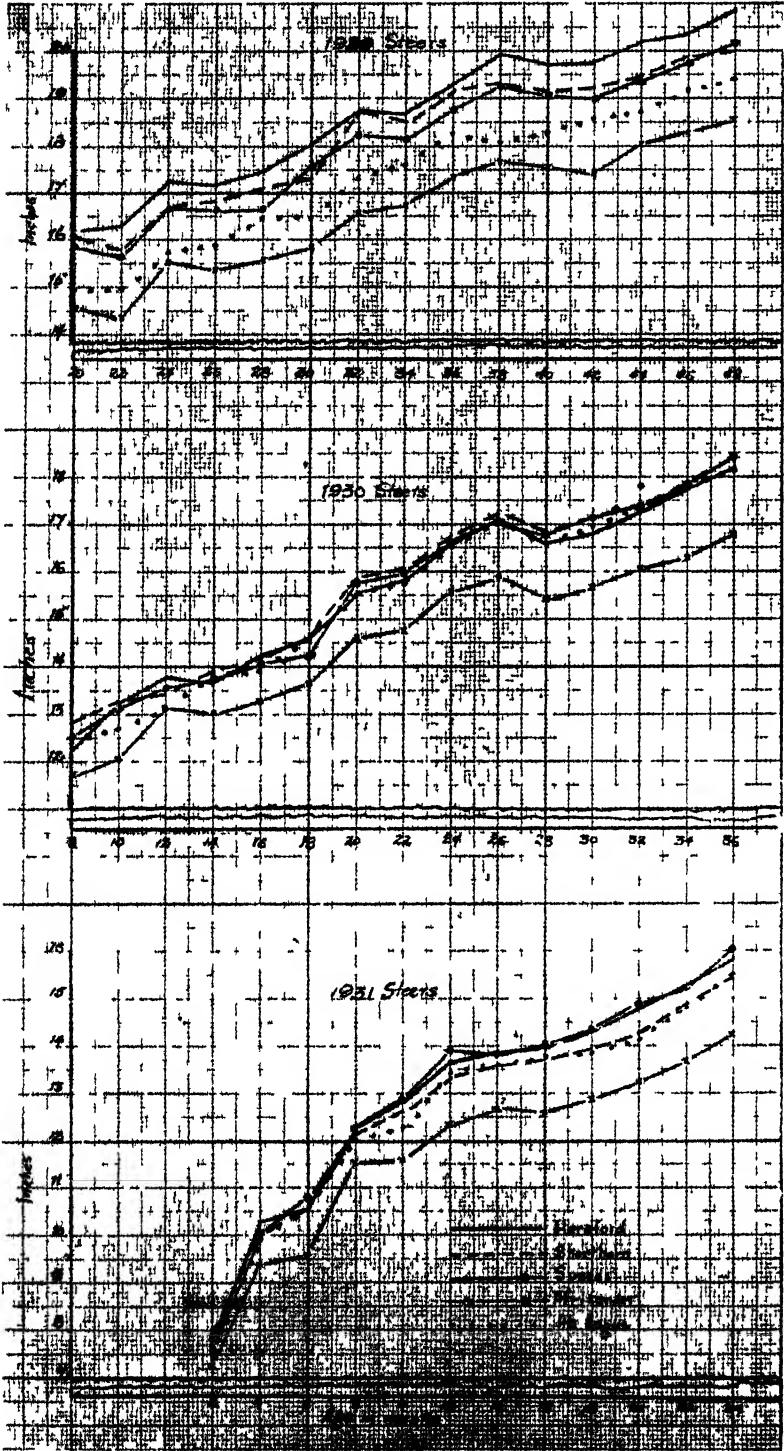


Fig. 23—Average growth curves of steers Width at hooks

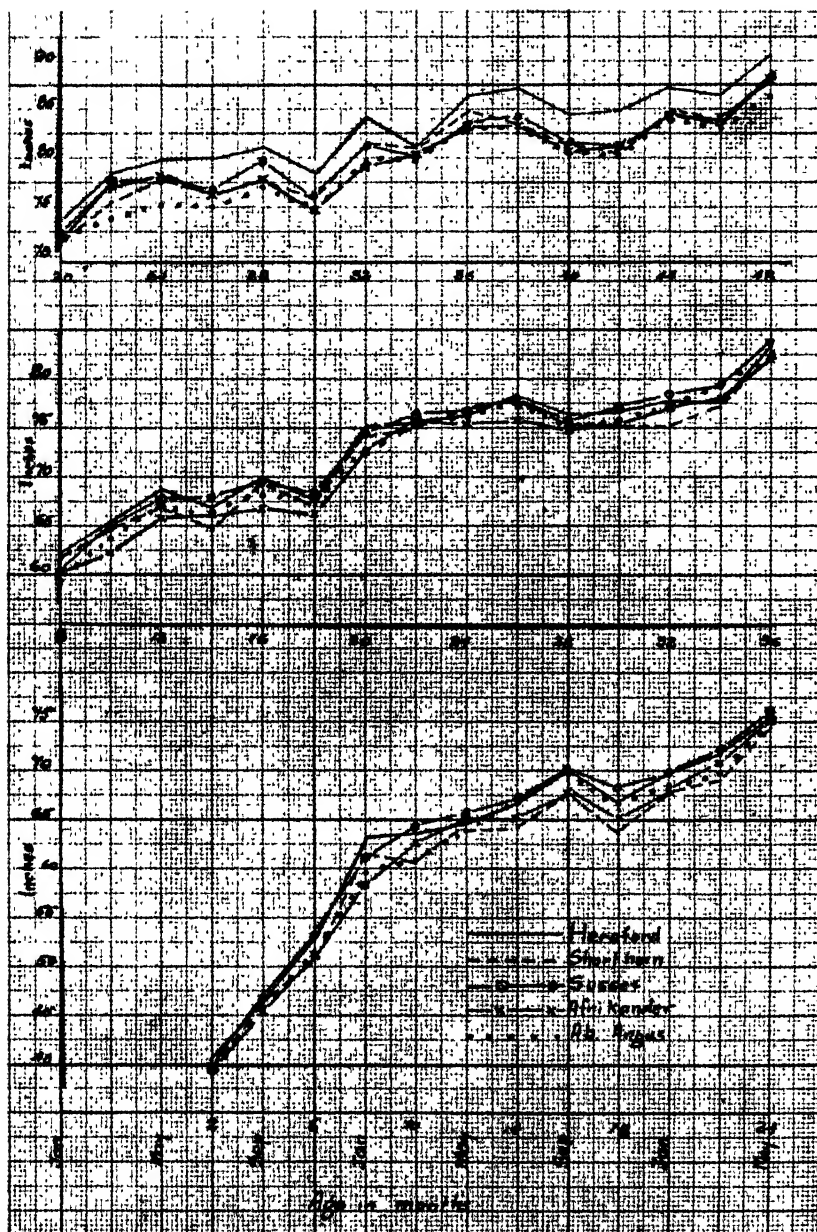
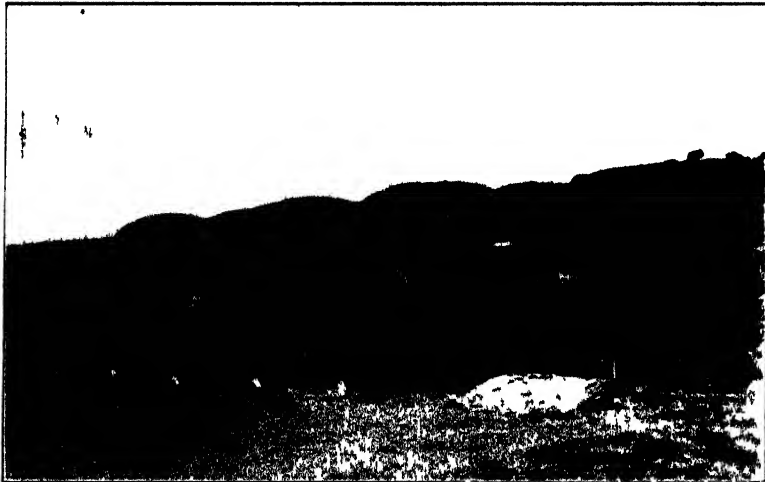


Fig. 24.—Average growth curves of steers. Paunch girth.



*Fig. 25* —Illustrating the type of country. Note dense bush and bare granite hills.

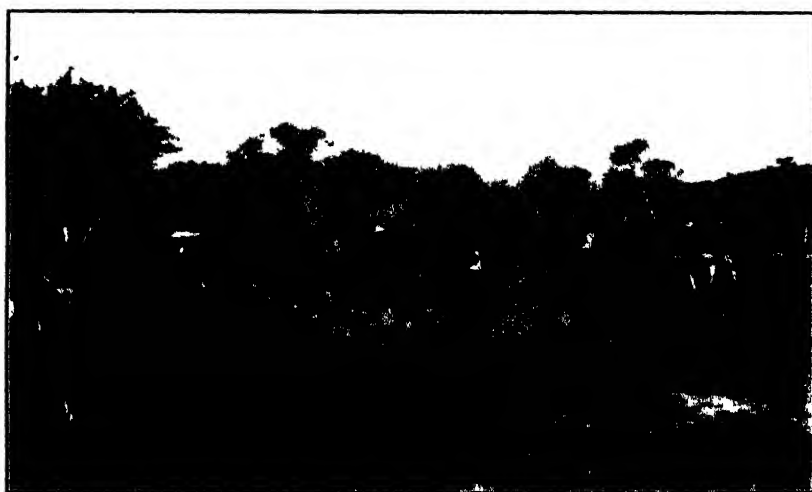
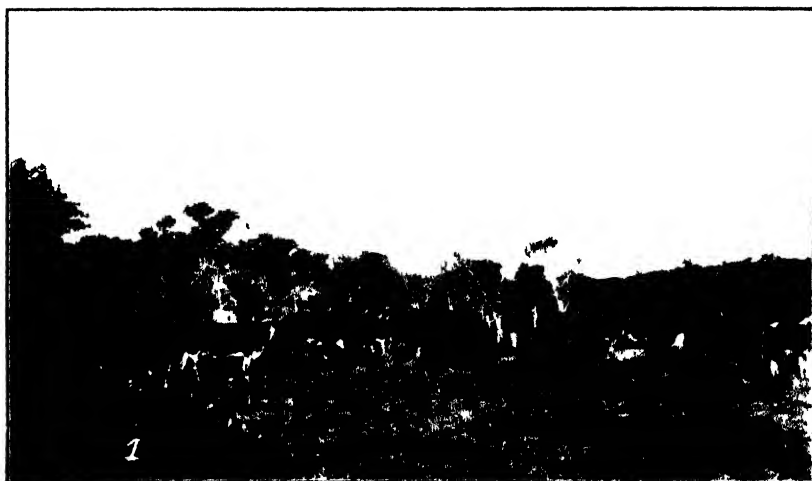


Fig. 26.—Type of unimproved cows used in the breeding operations.



Fig. 27.—Type of unimproved cows used in the breeding operations.



Fig 28.—Sires of the Hereford half-breds



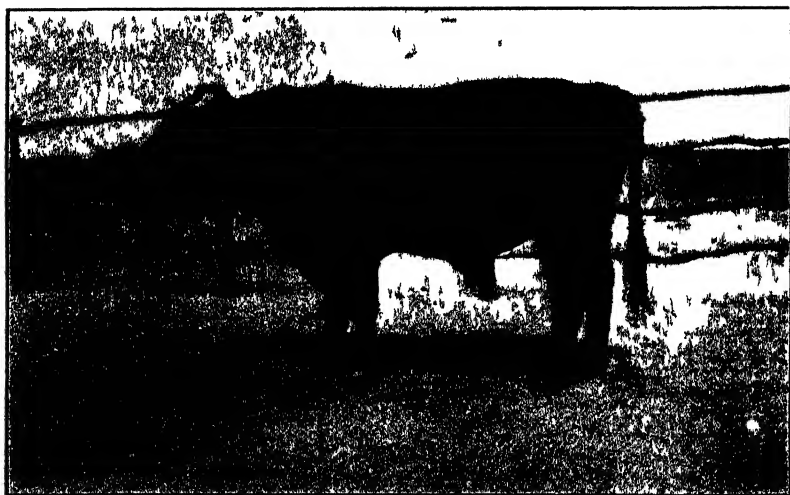
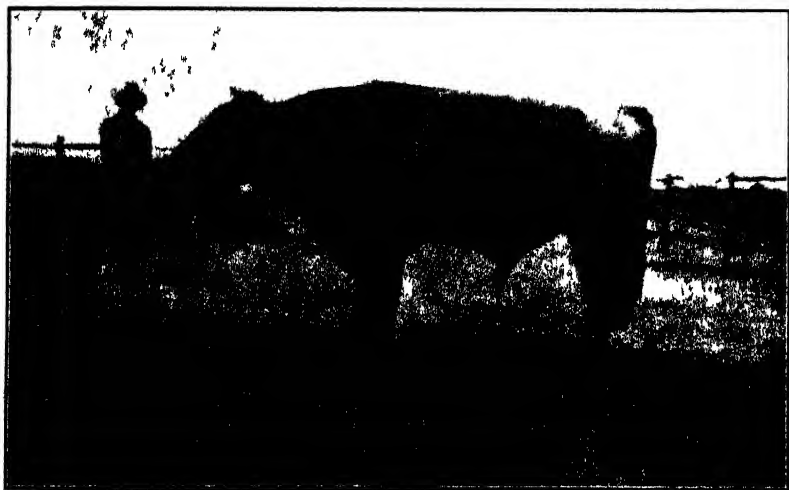


Fig 20.—Sires of the Shorthorn half-breds.

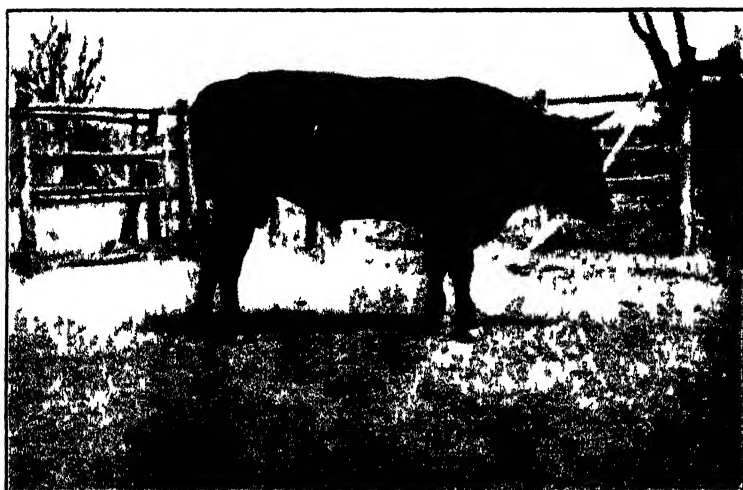


Fig 30 —Sires of the Sussex half-breds

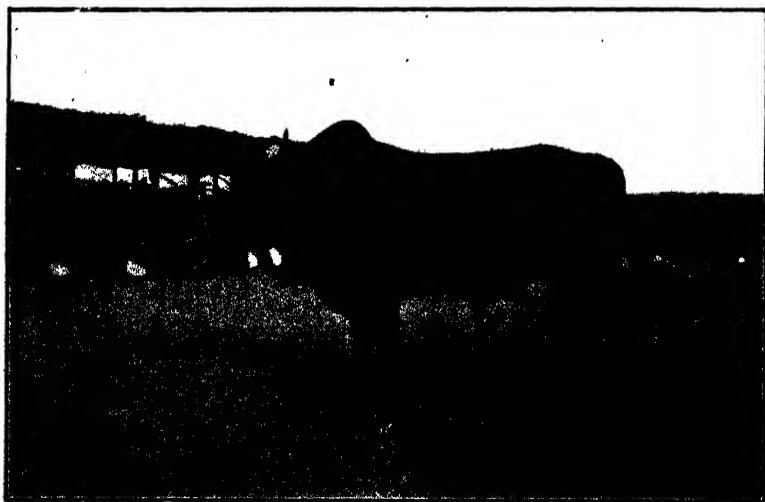
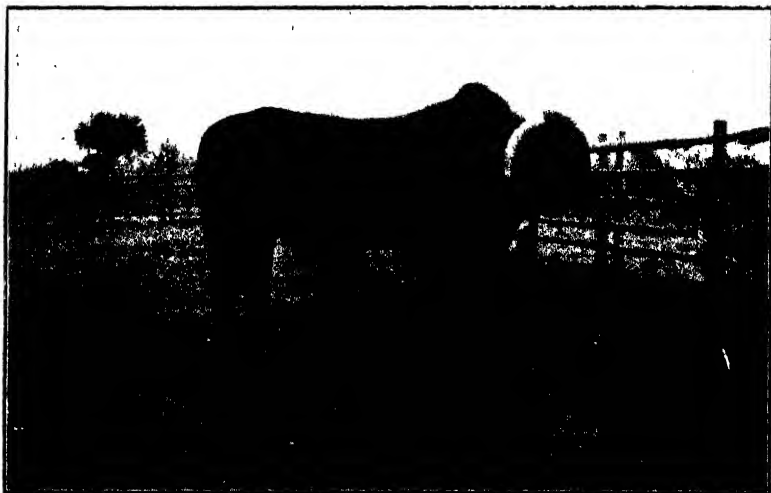
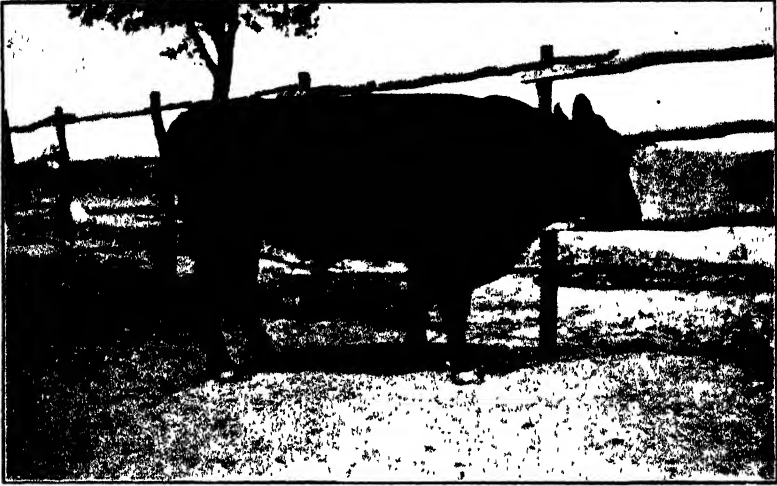


Fig. 31.—Sires of the Afrikaner half-breds.



*Fig. 32.*—Sires of the Aberdeen-Angus half-breds.



Fig. 33.—Hereford half-bred calves.



Fig. 34.—Shorthorn half-bred calves.



Fig 35 -Sussex half-bred calves.

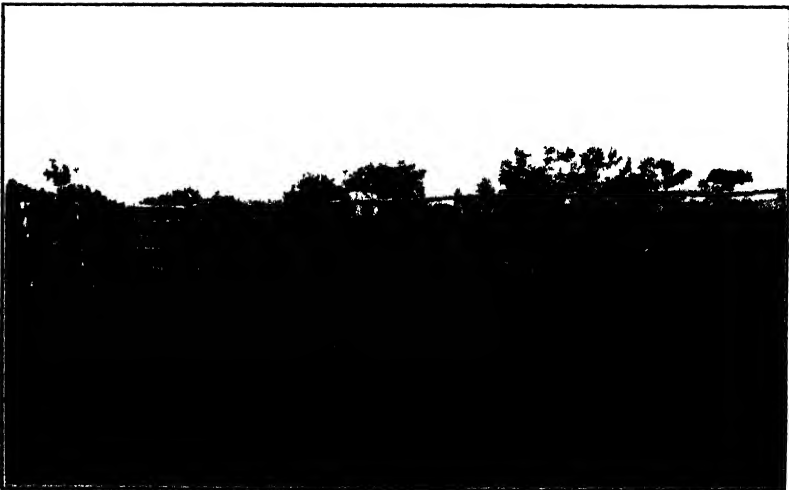


Fig. 36.—Afrikander half-bred calves.

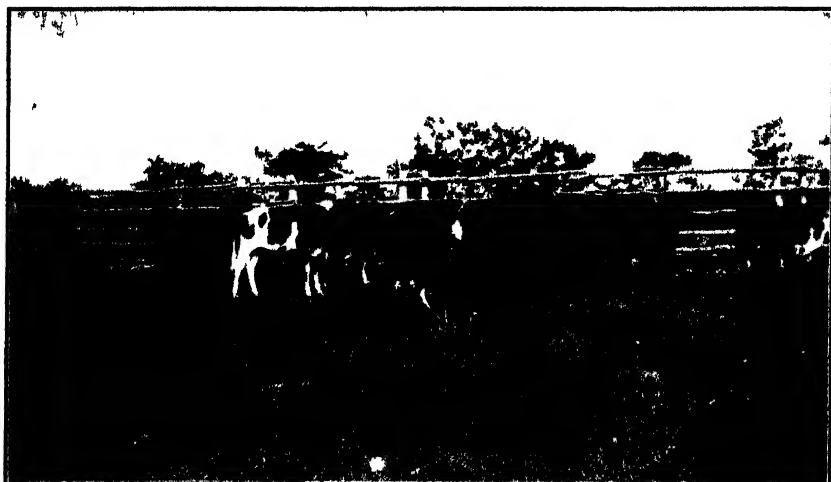


Fig. 37.—Aberdeen-Angus half-bred calves.



Fig. 38.—Hereford half-bred cow.

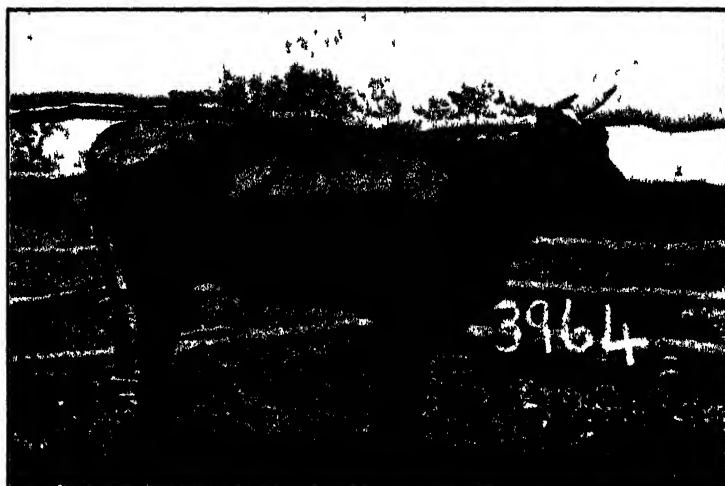


Fig. 39.—Shorthorn half-bred cow.



Fig. 40.—Sussex half-bred cow.





Fig. 41.—Afrikander half-bred cow.



Fig. 42.—Afrikander half-bred cow.

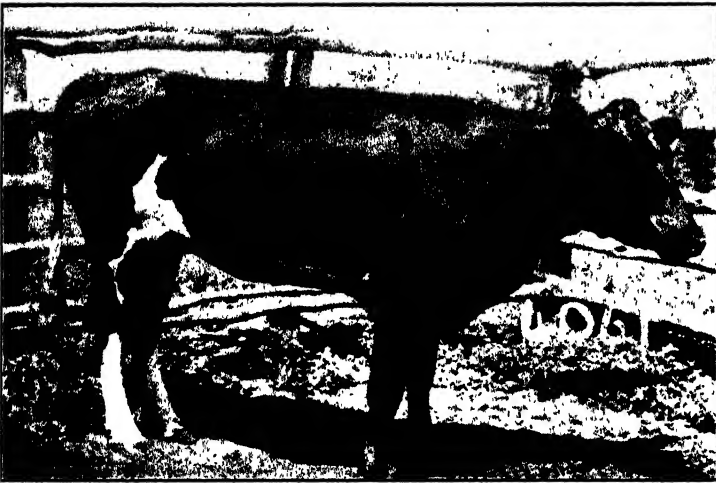


Fig 43.—Aberdeen-Angus half-bred cow.



## Section VIII.

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### Miscellaneous.

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## **Anatomical Study No. 60.**

### **Some Comments on the Hump of African Cattle.**

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., and  
J. H. R. BISSCHOP, B.Sc.(Agric.), B.V.Sc.,  
Veterinary Research Officers, Onderstepoort.

#### INTRODUCTION.

As shown in Curson and Epstein's paper (1934), the parent stocks of African cattle are believed to have been

- (a) *Hamitic Longhorn* with no hump,
- (b) *Brachyceros*, also with no hump,
- and (c) *Longhorned Zebu* (called *Afrikander* in South Africa) with a well-marked hump, which "does not fall abruptly on to withers, but slopes gradually" (Fig. 6).

Arising from the above are two chief intermixtures or types which have been described by Epstein (*The Origin of Africa's Indigenous Domestic Animals*, Chapter IV, in preparation) as follows:—

(I) *Shorthorned Zebu* (of many sub-types). This he considers to represent the influence while still in Asia of *Brachyceros* on the *Longhorned Zebu*. The type, like its longhorned relative, is also humped, but the structure differs in several respects from that of the *Afrikander* (Fig. 7).

And (II) *Sanga*.—Cattle of this type, occurring in Central and South Africa, are believed to be derived from the intermingling in North Africa of *Hamitic Longhorn* and *Longhorned Zebu* stocks. *Sanga* cattle, sometimes called *Zebus*, receive a separate section in Epstein's work referred to above. As in the case of the *Shorthorned Zebu* and *Brachyceros* types, the terms *Sanga* includes a large number of sub-types (Fig. 8).

# THE OBJECT OF THE STUDY.

While osteological evidence undoubtedly supports Epstein's contention, there are facts in regard to the soft structures, especially the hump, which call for further consideration. It is therefore the purpose of this work to record observations made on the various forms of hump<sup>(1)</sup>.

As will be seen from the subjoined extracts taken from another valuable contribution (*The Red Afrikaner* also in preparation), Epstein says comparatively little that is helpful regarding the hump:

(a) " Zebu, in its original form (*i.e.* Longhorned) is distinguished from other species by its hump which consists of fat and muscle tissues ". It " represents an accumulation of reserve materials similar to the steatopygy of the Bushman and Hottentot women, the fat tail of various sheep and the humps of the camel and dromedary . . . In animals living in steppes (of Asia) the accumulations appear on those parts of the body where they will not impede free movement. The humps of the Zebu cattle point therefore to a steppe country as the place of their evolution " (p. 63).

(b) " The penetration into Africa by Semitic nomads with their Zebu cattle " resulted in " contact with the longhorned cattle of Egypt which had accompanied the Hamites on their migrations to the west and south ". A consequence of this impact " as far as the cattle were concerned " were changes mainly affecting " the length of the horns and size of the hump " (pp. 63 and 64).

And (c) " The hump which appears in African cattle even more frequently than gigantic horns . . . is simply a product of cross-breeding<sup>(2)</sup>. The same result of variation as is obtained from the generation F2 onwards by crossing thin and fat tailed sheep is apparent in the formation of the hump in most African breeds of cattle. According to the degree of admixture of Zebu blood to the Hamitic cattle of primigenius type, the hump is developed to a greater or lesser extent, or often entirely missing " (p. 66). Indeed, there are breeds " in eastern, western, central and southern Africa, . . . which must be regarded as pure Zebus, although in some of these breeds the hump has almost entirely disappeared " (p. 66).

The chief points contained in the above are that the hump of the Longhorned Zebu (i) consists of fat and muscle tissues, (ii) became altered in size through admixture in North Africa with Hamitic Longhorn cattle (giving rise to Sanga cattle), and (iii) answers genetically in the same way as the crossing of thin and fat tailed sheep.

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(1) It must be emphasized that as far back as 1916 Hornby, H. E. (now Director of Veterinary Services, Tanganyika), had demonstrated that the hump of the Shorthorned Zebu was associated with the *M. rhomboideus*. He sent (letter 29/3/34) his notes to one of the authors (H. H. C.), and these have been placed in Departmental File 258/106.

(2) This statement obviously refers to the Sanga type,

C. Sheppard Cruz (1934), while not following the classification of Epstein, also does not consider that cattle of the Sanga type are "derived from near or remote progenitors of Afrikaner blood".

Our attitude is briefly thus. Whatever the origin of the Short-horned Zebu and Sanga cattle, there are certain facts with regard to the humps which should be emphasised, *viz.* (a) the marked resemblance of Loughorned Zebu and Sanga as would be expected in the light of Epstein's theory, and (b) the striking difference between the humps of the Loughorned Zebu and Sanga types on the one hand and the hump of the Shorthorn Zebu on the other.

Furthermore, in view of the fact that animal husbandrymen frequently speak of "attached", "loose", and "modified" humps, terms of little anatomical value, it is particularly necessary to make descriptions of the hump available.

#### EXTERNAL DESCRIPTION OF HUMP.

(a) The official description of the Afrikaner hump as published by the Afrikaner Cattle Breeders Society of South Africa (Bosman, A. M. 1932) is given thus:—"Large prominent and set closely to withers".

Viewed from the side the hump is situated in front of the withers. It occupies the posterior two-thirds of the upper border of the neck. The structure is somewhat pyramidal in shape and cranially it rises at an angle of about  $40^\circ$  to a rounded apex situated well in front of the point of the withers. From this rounded apex the hump falls at an angle of just over  $30^\circ$  on to the withers and merges with it to form a uniform and strong attachment.

Viewed from the front the hump appears to sit snugly over the upper border of the neck and is firmly attached to its sides, from which it rises steeply at an angle of about  $60^\circ$  to  $65^\circ$  to a rounded summit. For this reason the hump of the Afrikaner is sometimes spoken of as being well attached.

In the bull (Fig. 6) the hump is decidedly prominent, rising sometimes to 8 inches above the top-line. In oxen the structure is a little less prominent and in females it is relatively smaller still. The hump is well developed even in a foetus.

(b) Epstein (*The Origin of Africa's Indigenous Domestic Animals*) in referring to the Sanga type states that "the tendency to be humped is more general (than the tendency to gigantic horns). It is true that the hump is not equally well developed in all Sanga breeds. In some it almost reaches the size of the humps of Indian Zebu's (i.e. of Shorthorned type) . . . in others again, as in the cattle round Lake Chad, the majority are entirely humpless and only individual animals are humped".

As shown above, he believes that "according to the degree of admixture of Zebu blood to the Hamitic Cattle . . ." so is "the hump developed to a greater or lesser extent, or often entirely missing".



It is evident therefore that there is a wide variation in the size of Sanga humps, but as a typical example we may take the Ambo beast (Groenewald and Curson 1933 and Bisschop and Curson 1935). In the former paper the hump was described (in the cow) as being "small and set well forward on withers". In the latter it was described (in Bull 5010) as follows:—"Situated in front of the withers and is well defined. Its anterior axial border passes up from the upper border of the neck to the apex of the hump at an angle of approximately  $40^{\circ}$ . From the apex to the withers the posterior axial border falls at an angle of about  $30^{\circ}$  to the horizontal. The hump is on the small size and is well attached to the neck".

It is thus evident that apart from size, the similarity in external appearance of the hump of the Afrikander and Ambo breeds is striking.

(c) The hump of the *Shorthorned Zebu* (Fig. 7) is less constantly pyramidal than in the two previous types. Very frequently the structure is not only more prominent but also actually dome-shaped. Another common feature is the greater mobility of the hump especially the posterior part due to its fatty nature. Most striking of all features is the *more caudal situation* of the hump, a vertical line through the summit passing at least through two vertebrae behind a line similarly drawn in the Afrikander or Sanga types. In extreme cases of hump development the term "loose" may be applied, but it must be emphasised that anatomically it is nevertheless securely attached, particularly by the *M. trapezius*, as will be indicated subsequently. The size of the hump varies greatly.

In the description of Indian Zebras apart from a statement that "the hump is well developed in the bull" (Gunn 1909), no detailed account is available. Hornby writes (letter L/48/49 of Nov. 17th, 1934) "that a Zebu foetus has a well-marked hump".

#### SOURCE OF MATERIAL FOR STUDY.

Apart from the illustrations which accompany this study, the authors have made observations on native types of cattle throughout Southern and Eastern Africa.

For dissecting purposes the source of material is also indicated in the explanations accompanying the various figures; but a special word of appreciation is due to Mr. H. E. Hornby, who sent Zebu (Shorthorned) humps from Tanganyika. To summarise, the anatomical observations were made on yearling bulls 5572 and 5736 (Afrikander), two humps of Shorthorned Zebu calves from Tanganyika, and the Ambo bull 5010 as representing the Sanga type. A Friesland bull obtained locally was also dissected and the features were similar to those shown in Figs. 9 and 13.

DESCRIPTION OF THE *Mm. Trapezius* AND *Rhomboideus* IN AN  
UNHUMPED ANIMAL (E.G. FRIESLAND).

As the above muscles are primarily associated with hump formation, it is advisable to describe them in the unhumped beast. Thereafter the features of the humped types will be tabulated for comparison.

*M. Trapezius.*

As in the horse this is superficial and fan-shaped. Its fibres extend from the dorsal aspect of the cervico-thoracic region (atlas to about the tenth thoracic vertebra) to the scapula. As they descend, caudally from the cervical region and cranially from the thoracic, they converge and terminate in an aponeurosis, which is inserted into the *spina scapulae*. The cervical and thoracic portions are, however, not clearly separated. The attachment of the fibres along the mid-dorsal line becomes more intimate as one follows the origin of the muscle backwards. The cranial border is firmly adherent along its anterior half to the *M. cleidooccipitalis*, and posteriorly to the *M. omotransversarius*. The caudal border is attached by fascia to the *M. latissimus dorsi* which in this region is covered by the sheet-like *M. cutaneus scapulae et humeri*.

*M. Rhomboideus.*

This muscle may be divided into a *pars cervicalis* and a *pars thoracalis*, the former being most conspicuous and having fibres pursuing generally a longitudinal direction, whereas the latter is relatively insignificant and has fibres running obliquely downwards and backwards. The origin is the *pars occipitalis* of the *ligamentum nuchae* and its caudal prolongation, the *ligamentum supraspinale*, while the insertion is the medial surface of the *cartilago scapulae*. The cervical part is pointed cranially, but as one proceeds caudally so does the muscular tissue become expanded.

In the bull, even of the *Brachyceros* type, the above muscles as well as the *M. splenius* and *M. serratus ventralis*, are well developed, but it is only in special circumstances e.g. raising the head that a hump-like structure is obvious and when evident, it is always to be found in the *anterior* cervical region. It will be observed (Fig. 5) that no such hump-like elevation is apparent, but a definite dome-like structure would be quite evident if the head were elevated. This clearly muscular structure is strictly speaking not a hump in the usually accepted sense of the word, and is sometimes referred to as the crest.

An account having been given of the "humpless" beast, we may now tabulate the anatomical features encountered in the humped types.

COMPARATIVE TABLE CONCERNING HUMPS OF AFRICAN CATTLE TYPES.

Feature.	Yearling Afrikaner Bulls (5572 and 5736).	Shorthorned Zebu <i>e.g.</i> Tanganyika, (See Figs. 48 and 49, Duerst). <sup>3</sup>	Sanga bull <i>e.g.</i> Ambo 5010 (2 yrs. 4 months.).
<i>M. trapezius</i> .....	The muscle is well developed and may be arbitrarily divided into cervical and thoracic parts, the former overlying the hump and the latter caudal of the hump. (See Fig 10.)	The muscle is distinctly separable into a cervical and a thoracic portions, the latter being darker in colour and more strongly developed. The cervical portion instead of arising entirely from the funicular part ( <i>pars occipitalis</i> ) of the <i>ligamentum nuchae</i> is closely associated with hump development. In fact it may be considered as a superficial cranio-lateral anchor or attachment of the hump. The thoracic portion is well developed and except for a small cranial slip does not act as a stay. (See Fig 11.)	Here it is also arbitrarily divisible into cervical and thoracic portions. There is no development of adipose tissue resembling the hump of the Shorthorned Zebu. (See Fig 12.)
<i>M. rhomboideus</i> .....	The <i>M. rhomboideus</i> has distinct cervical and thoracic portions. The cervical part is extraordinarily well developed, and forms the hump. The thoracic part is made up of short muscular fibres having generally a ventro-caudal direction. (See Fig. 14.)	This is characterised by the entire absence of the longitudinally arranged cervical part (*). Instead, the anterior portion of the thoracic part is very much developed, in fact the fatty hump having not only a foundation, but also a framework of muscular tissue. The fibres run generally in a ventro-caudal direction, especially at the base of the hump. This structure is best described as a musculo-adipose development of the <i>M. rhomboideus</i> , <i>pars thoracalis</i> (anterior portion). (See Fig 15.)	The muscle is clearly represented as a cervical portion and as a thoracic portion. The former is markedly enlarged, in fact constitutes the hump. The fleshy fibres are disposed as a rule in a longitudinal direction. The thoracic part is relatively poorly developed and its fibres run in a ventro-caudal direction. (See Fig. 16.)
Situation of hump.....	The hump lies cranial to the <i>angulus cranialis</i> of the scapula over the 6th/7th cervical to 4th/5th thoracic vertebrae ( <i>Crista spinosa</i> )—a cervico-thoracic hump.	Dorsal to the <i>margo vertebralis</i> of the <i>cartilago scapulae</i> . In terms of vertebrae it lies over the first to ninth thoracic vertebrae ( <i>Crista spinosa</i> )—a thoracic hump.	From the sixth to seventh cervical vertebra caudally to the 4th/5th thoracic bone. The hump is definitely cranial to the <i>angulus cranialis</i> of the scapula—a cervico-thoracic hump.

(\*) Two specimens, probably from bull calves, were kindly sent by Mr. H. E. Hornby, Director of Veterinary Services, Tanganyika.

(†) Since the cervical and thoracic parts of the *M. rhomboideus* are not separated by any well defined structure, *e.g.* of fibrous nature, it is of course possible to consider the hump of the Shorthorned Zebu as a development of the part posterior of the cervical part of *M. rhomboideus*. Duerst (1931, p. 46) considers that a fusion of the two parts has taken place.

## DISCUSSION.

Epstein apparently believes that the hump of the Longhorned Zebu (Afrikander) represents an accumulation of reserve material as seen in the steatopygy of certain native women, the fat deposits of certain native sheep and the humps of the camel and dromedary. It is clear from our observations that the comparison is not applicable, for whereas in the cases of fat accumulation there is a storage of reserve energy *per se*, in the Afrikander this is not so. The same remark applies to the hump of the Sanga type.

Epstein is, however, perfectly correct if he extends the comparison to the hump of the Shorthorned Zebu, for here definitely there is fat storage. Hornby's comments, which would support Epstein, are as follows: "At birth the musculature is well marked, but as the animal develops this becomes more and more obscured by fat deposition. Even when the adult loses condition and the hump shrinks very greatly, the hump never regains the lean muscular state of calfhood—a certain amount of fat and fibrous tissue persist". (Letter L/48/49 of November 17th, 1934.)

In the description of the *M. rhomboideus* of the Shorthorned Zebu, reference is made to the muscular tissue acting as a framework of the hump. This disposition is well shown in Fig. 18, which should be compared with Figs. 17 and 19.

In the Afrikander and Sanga cattle, fat may also occur, but it is usually distributed in layers, first subcutaneous, then between the *M. trapezius* and *M. rhomboideus*, and finally beneath the latter. In an adult Afrikander bull the hump may weigh from Kg. 10 upwards.

From a perusal of the tabulated statement along with the corresponding figures, it is clear that (a) there is a great similarity both in situation and structure of the hump of the Longhorned Zebu and Ambo, and (b) there is a marked difference between the above humps and that of the Shorthorned Zebu. The apparent difference in the arrangement of the muscular fibres of the Longhorned Zebu and Ambo need not be seriously considered, for in the hump of Afrikander yearling bull 5572, the arrangement resembled that of the Ambo. It is of course evident that the drawings are diagrammatic and not to scale in view of the widely divergent ages of the subjects. See, however, Figures 17-19.

Relatively little seems to be known concerning the significance of the hump from an evolutionary point of view. While the intermixture of the Longhorned Zebu and Hamitic Longhorn undoubtedly gave rise to the Sanga type, the position is not so clear in regard to the Shorthorned Zebu. The importance of further investigation in this problem is emphasised.

## CONCLUSION.

The result of this study suggests that humps may be classified:

- (a) According to situation—cervico-thoracic and thoracic.
- (b) According to structure—muscular and musculo-fatty.
- (c) According to function—traction (locomotion) and storage of reserve fat.

It is interesting that some cattle have humps and others not.

The similarity of the humps of the Afrikander and Sanga types, being cervico-thoracic and muscular is noteworthy in view of Epstein's theory that the latter is derived from the intermixture of the Longhorned Zebu and Hamitic Longhorn. The dissimilarity between the above humps and that of the Shorthorned Zebu is striking. As just stated little is known regarding the significance of the hump from an evolutionary point of view.

#### ACKNOWLEDGEMENTS.

We appreciate the gift of material by Mr. H. E. Hornby, Director of Veterinary Services, Mpapwa, Tanganyika, the co-operation of Dr. J. Botelho, Director of Veterinary Services, Mozambique, and his assistant, Dr. Sheppard Cruz, both of Lourenço Marques, and the use of Dr. H. Epstein's unpublished work on native cattle.

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#### APPENDIX<sup>(5)</sup>.

##### USEFUL REFERENCES IN STUDYING AFRICAN CATTLE.

###### AFRIKANDERS.

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2. HOLM, A. (1912). Afrikander Cattle. *Agr. Jl. Union of South Africa*, IV, p. 687, Nov.

<sup>(5)</sup> For previous lists see Appendix 3 of Groenewald and Curson (1933) and the Appendix in Bisschop and Curson (1935).

3. HOLM, A. (1916). Report on Cattle Feeding Experiments. *Bull.* 15 of 1916, *Dept. of Agr.*
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11. FINLAY, G. F. (1925). *Cattle Breeding. Proceedings of the Scottish Cattle Breeding Conference. Edinburgh, 1934.* Oliver and Boyd, Edinburgh. Chap. XXXIII.
12. REINECKE, T. G. W. (1931). Africander Cattle. *Farming in South Africa*, VI (64), July.
13. BOSMAN, G. J. (1928). A Grand Old Man in Africander Cattle Breeding. *Farming in South Africa*, III (29), Aug.
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#### ADDENDUM.

A copy of Anatomical Study, No. 60, was submitted to Dr. H. Epstein who, in a letter to the Director of Veterinary Services, dated 1st August, 1935, made the following comments:—

“ Their (i.e. Messrs. Curson and Bisschop) conclusions lead to the question whether the similarity of the humps of the Afrikander and Sanga types proves the former also a Sanga breed. On the basis of osteological comparison I am still inclined to deny this. Curson and Bisschop also seem to deny it, stating on p. 627 ‘The intermixture of the Longhorned Zebu and Hamitic Longhorn undoubtedly gave rise to the Sanga type’ and on p. 623 ‘certain facts which should be emphasised, viz. (a) the marked resemblance of Longhorned Zebu and Sanga as would be expected in the light of Epstein’s theory’. It, however, this similarity does not prove that the Afrikander carries Egyptian Longhorn blood, but merely that cattle of Afrikander type have contributed to the evolution of the Sanga, the dissimilarity between the above humps and that of the Shorthorned Zebu calls for an explanation.

It is possible that, after the African Longhorned Zebu had branched off the main stem, a few thousand years more of domestication under the climatic conditions of Asia have led there to a gradual change (higher evolutionary specialisation?) in the structure of the Zebu hump.

Investigations of the foetal humps (\*) of Afrikanders and Shorthorned Zebus would probably answer this question, since it can be taken for granted that, if the hump of the Shorthorned Zebu was originally also cervico-thoracic and muscular, this would appear in one of the foetal stages.

The second conclusion—one which I favour less than the former—is that the influence of *Brachyceros* blood on the Zebu may have given rise to a change in the hump structure. To investigate this, it would be necessary to compare the humps of Shorthorned Zebus with those of the rare longhorned breeds occurring in southern India. The picture of the skull of such a beast resembling the Afrikander skull to an extraordinary degree, is produced by Duerst in his *Animal Remains from the Excavations at Anau*. I do not wish to imply that long or short horns in Zebus have any relation to the structure of the hump. I merely want to state that in some less accessible parts of southern India longhorned Zebu breeds may exist that have preserved their original structure for thousands of years, just as the Afrikanders' ancestors have. But I realise that it would be very difficult to carry out such investigations.

Finally, both factors combined, the long period of domestication in Asia and the influence of *Brachyceros* cattle, may have caused the change in the structure of the hump."



Fig. 1.—African *Brachyceros* Cow and Bull Calf. (Curson and Epstein 1934.)  
[Photo: Dr. P. J. du Toit.]

(\*) Mr. Hornby, on being approached as to whether the foetus of a Short-horned Zebu had a thoracic or cervico-thoracic hump, replies (letter L 250/10 of 25/11/35): "I have just had a look at a new-born slightly premature Zebu calf. There was a definite though small hump which was thoracic in position. It was also musculo-fatty in that although no actual fat was present, the course of the muscle fibres was not clear". The Afrikander foetus definitely has a cervico-thoracic hump of muscular structure.

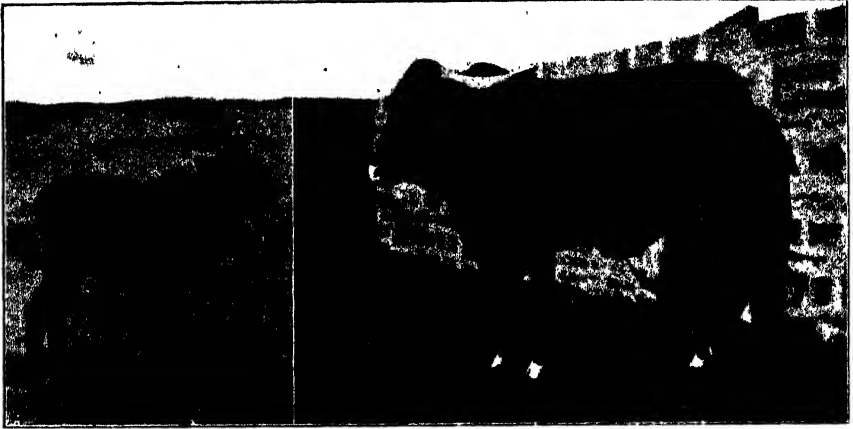


Fig. 2.—Longhorned Zebu—Africander—Cow and Bull Calf. (Epstein 1933.)



Fig. 3.—Shorthorned Zebu—Masai Ugogo—Cow and Bull Calf. (McCall 1928.)



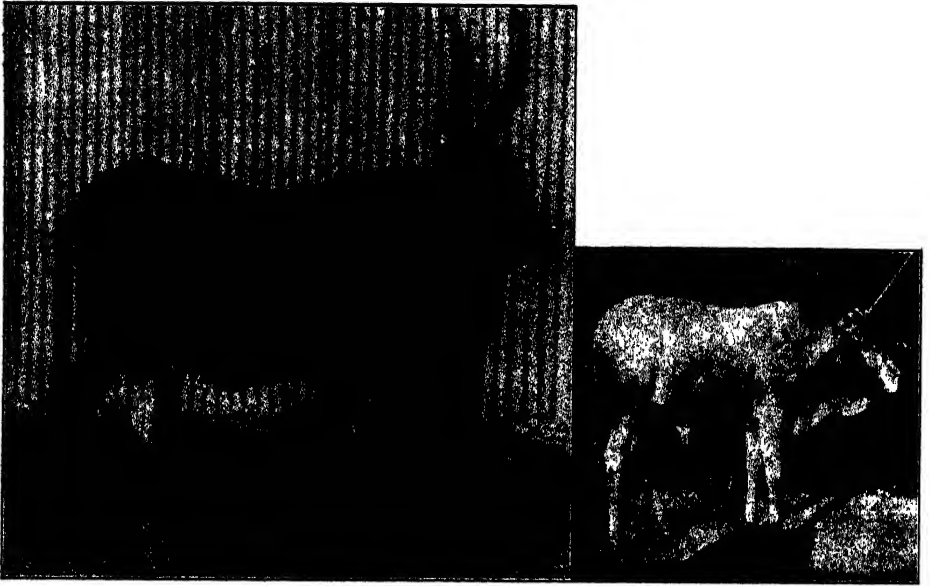


Fig. 4.—South African Sanga—Ambo—Cow 3586 and Bull Calf 5010, 7 months.  
(Groenewald and Curson 1933.)

[Photo: T. Meyer.]

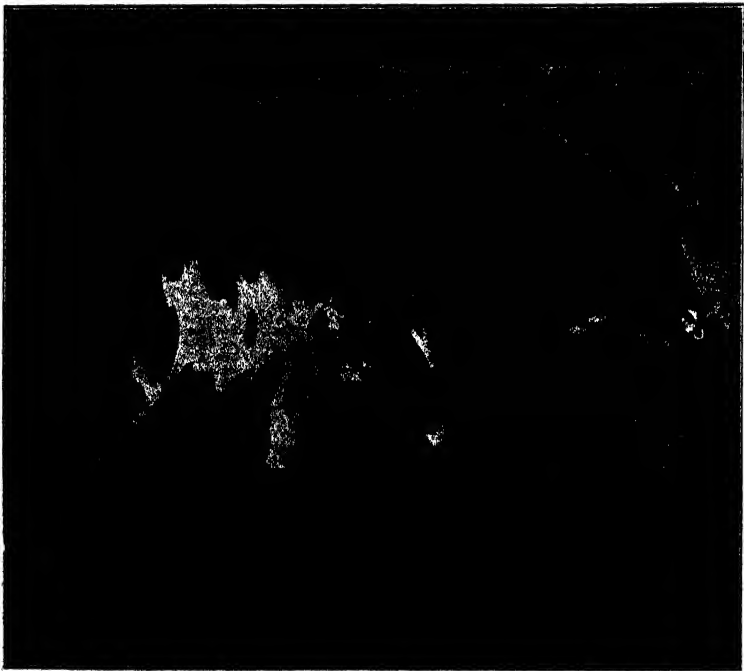


Fig. 5.—African Brachyceros Bull, 34 years, weight 350 lb., beside pure-bred  
Lincoln Bull. (Henderson 1928.)



[Photo: T. Meyer.

Fig. 6.—Longhorned Zebu—Afrikander—Bull 4327.



Fig. 7.—Shorthorned Zebu—Golden Dun Mkalama—Bull, 2 years. (McCall 1928.)



[Photo: T. Meyer.

Fig. 8.—South African Sanga—Ambo—Bull 5010.



Fig. 9.—Cow of European *Brachyceros* type. Figure taken from Martin's *Anatomie der Haustiere*, iii Band, Fig. 131, page 388d. There is no hump. Superficial Dissection.



Fig. 10.—Longhorned Zebu—Afrikander—Bull 5786, showing cervico-thoracic hump with skin removed but *M. trapezius* intact.



Fig. 11.—Shorthorned Zebu—Tanganyika—Bull Calf (Hornby), showing *thoracic* hump with skin removed but *M. trapezius* intact.

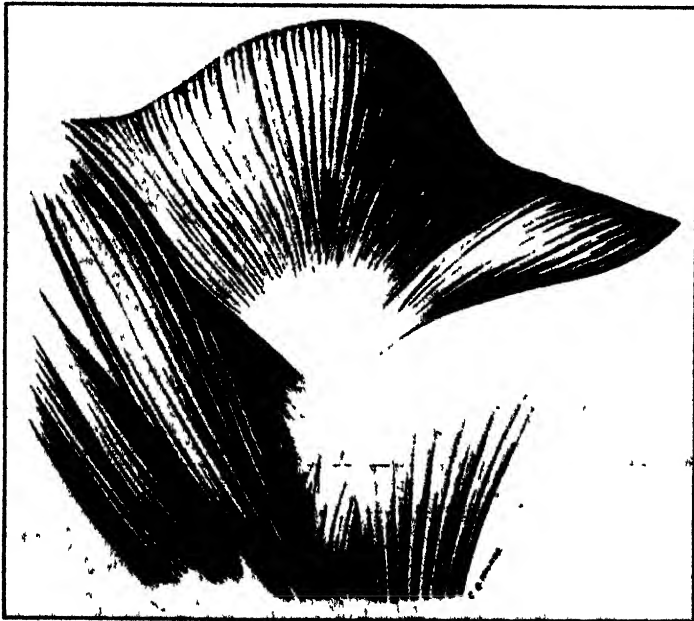


Fig. 12.—South African Sanga—Ambo—Bull 5010, 2 years 4 months, showing *cervico-thoracic* hump with skin removed but *M. trapezius* intact.



Fig. 13.—Cow of European Branchyceros type. Figure taken from Martin's *Anatomie der Hauttiere*, iii Band, Fig. 132, p. 238e. There is no hump—deep dissection.



Fig. 11.—Longhorned Zebu—Afrikander—Bull 5736, showing *cervico-thoracic* hump after removal of *M. trapezius*.



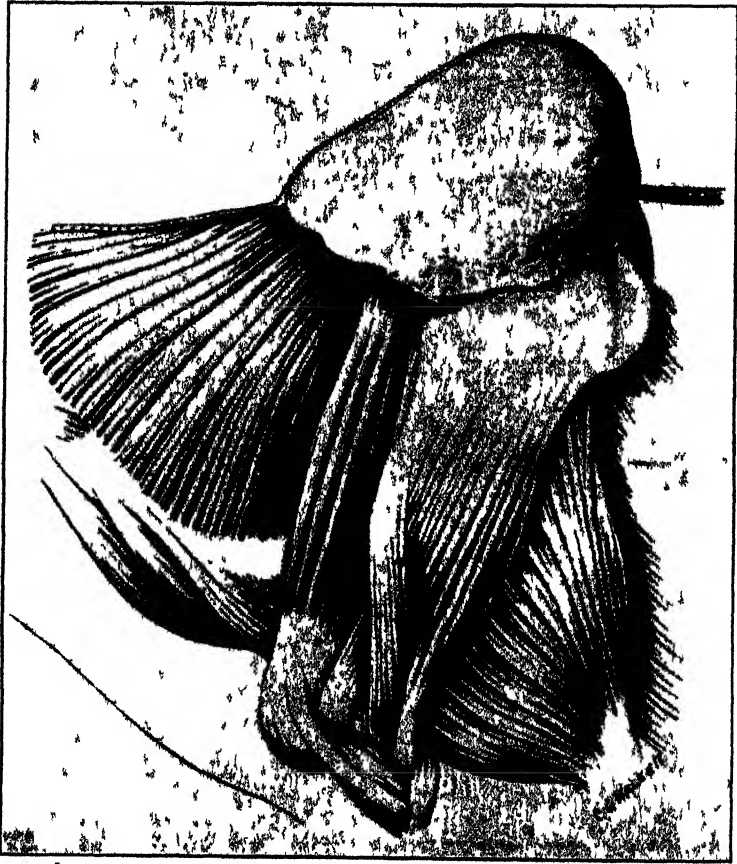


Fig 15.—Shorthorned Zebu—Tanganyika—Bull Calf (Hornby), showing thoracic hump with *M. trapezius* removed. It will be observed that the *M. rhomboideus* is quite different from that in *Brachyceros*, Longhorned Zebu or South African Sanga type (e g Ambo).



Fig. 16.—South African Sanga—Ambo—Bull 5010, showing cervico-thoracic hump itself with *M. trapezius* removed.

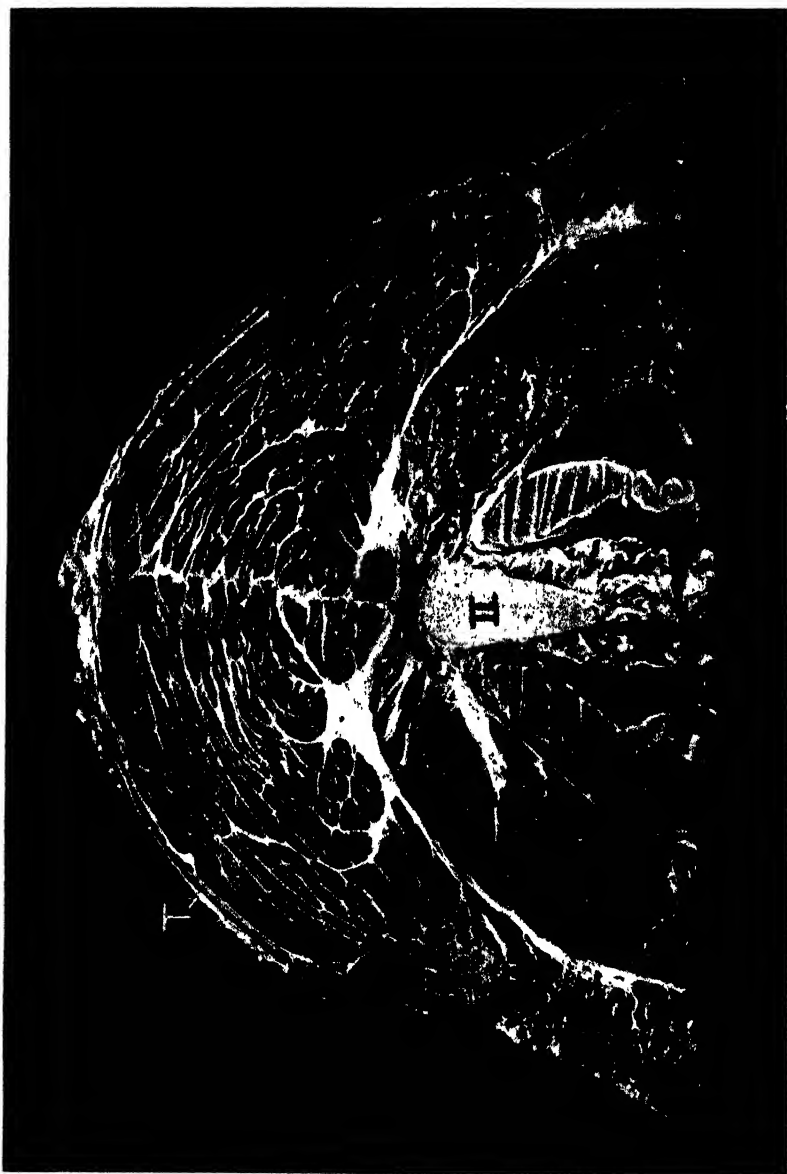


Fig. 17.—Transverse section (at summit) of hump of Longhorned Zebu—Afrikander—Bull 5572 over 2nd thoracic vertebra (II) T=*M. trapezius*; R=*M. rhomboideus*; S=*M. serratus ventralis*; SP=*M. splenius*, N=*L. nuchae*. Cranial View,  $\frac{3}{4}$  Natural size.

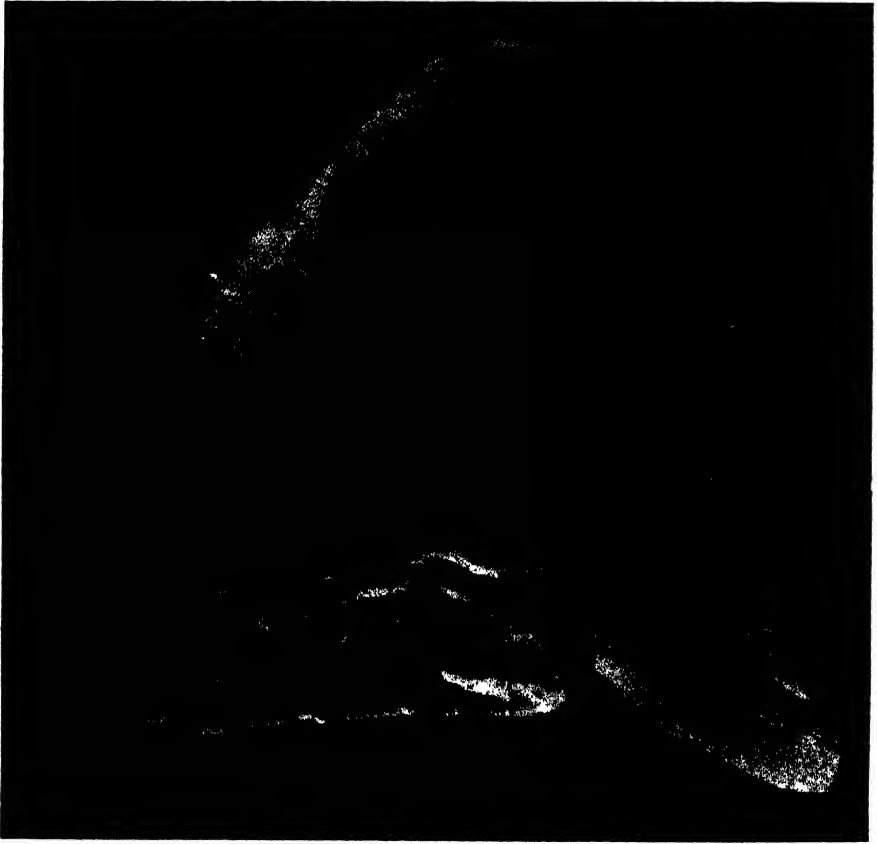


Fig. 18.—Transverse section (at summit) of hump of Shorthorned Zebu, Bull Calf (Hornby), over 4th thoracic vertebra. (IV). Distorted appearance due to tight packing in drum. Cranial view.  $1\frac{1}{2}$  Natural size.

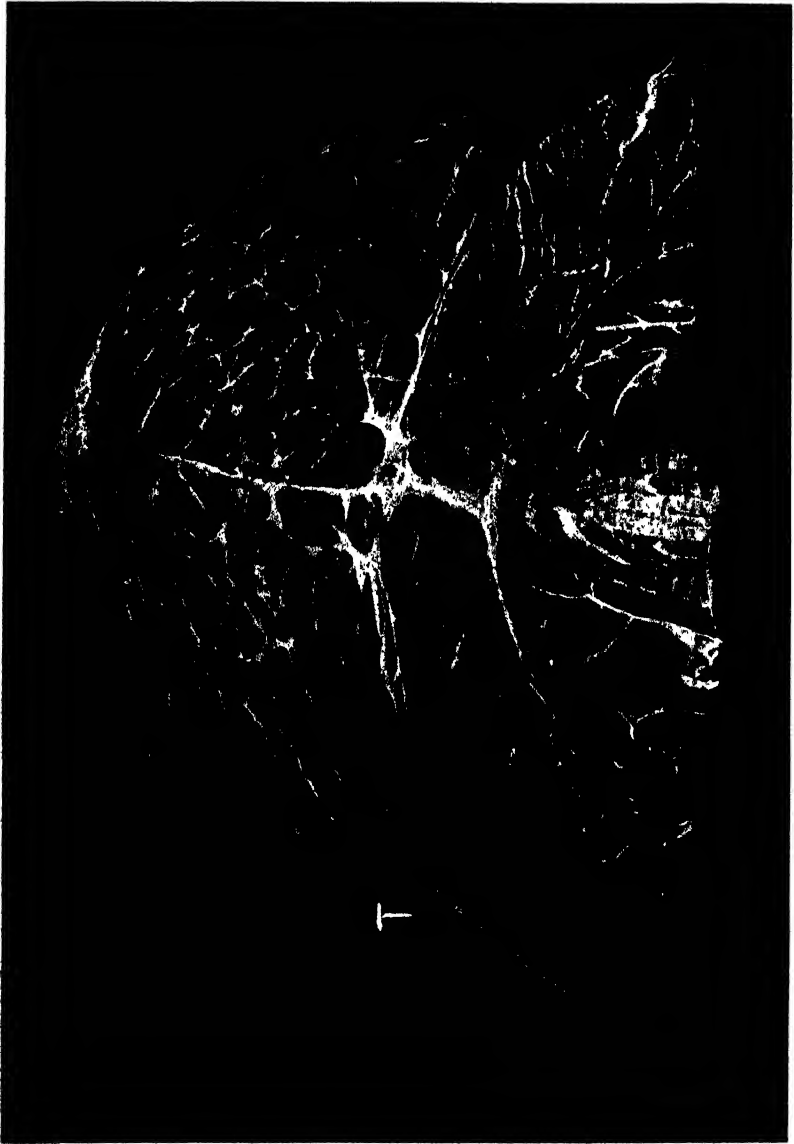


Fig. 19.—Transverse section (at summit) of hump of South African Sanga—Ambo—Bull 5010 over approximately 2nd thoracic vertebra. Cranial view.  $\frac{1}{3}$  Natural size. Letters have same meaning as in Fig. 17.

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